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THE DEVELOPMENT AND GERMINATION OF THE INTRAEPIDERMAL TELIOSPORES OF MELAMPSORELLA CERASTII[1]

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(WITH 30 FIGURES)

The intraepidermal teliospores of the Pucciniastreae are of interest because of their peculiar habit of growth and wide variations which exist in the shape, size, and number of cells largely as the result of spatial relationships within the epidermal cells of the hosts. Since 1933 the writer has studied several species of these rusts and their manner of development has been ascertained. These include *Calyptospora goeppertiana, Milesia polypodophila, M. intermedia, M. fructuosa, Thekopsora vacciniorum* (**5**), *M. marginalis* (**6**) *and Hyalopsora aspidiotus* (**7**). This paper presents information on the development and germination of the teliospores of the closely related genus *Melampsorella.* Olive (**4**) has recently described the development and germination of the teliospores of *Thekopsora hydrangeae* in connection with a detailed study of the ontogeny of the sori on both the aecial and telial hosts. His account agrees in general with the method previously described, particularly with *T. vacciniorum,* but differs in some de-

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tails. A comparison of the results herein reported with those previously described, and particularly with *T. hydrangeae,* will be found later in this paper.

MATERIAL AND METHODS

Uredinial and telial material of *Melampsorella* was collected first on July 3, 1942, on a few plants of *Cerastium* sp. and *Stellaria* sp. in the Medicine Bow National Forest, Wyoming, in the vicinity of the University of Wyoming Summer Camp, at an altitude of 10,000 ft. On July 15 and subsequently it was found abundantly on *C. arvense* in a small area in this same general locality. The description of teliospore development and germination is based upon material on this host.

This material has been identified as belonging to the species *M. cerastii* (Pers.) Schroet. The status of the species of this genus is rather confused at the present time. Although Arthur (**2**) has made a single species, the writer has pointed out (**8**, **9**, **10**) that there are such differences on the aecial hosts in the type of pycnia, in the size, color, and markings of the aeciospores, as well as in the size and type of broom produced, that there are two species. In an attempt to prove this, two sets of infection experiments were undertaken, the first to obtain uredinial and telial material from aeciospores from infections on *Picea* and *Abies*. Some measure of success was obtained here; out of ninety sets of parallel inoculations, thirteen successful transfers were made with aeciospores from *Abies lasiocarpa* to *Cerastium arvense, Stellaria longipes,* and *S. umbellata.* From *Picea,* infections were negative, except in one doubtful case where a single plant of *S. longipes* became infected. The second set of inoculations were from telial material to *Picea* and *Abies,* half of the leaves of one plant being used on the former, the remainder on the latter. Results from this have so far been negative. Weir and Hubert (**12**), however, were successful in obtaining infections from aeciospores from *Picea* to *Stellaria borealis* and *S. longipes.*

The writer has examined and measured many urediniospores on the Caryophyllaceous hosts from his own collections and from the Arthur Herbarium, and has not been able to find any differences. The intradermal teliospores are, unfortunately, not suitable for tax-

onomic work because of their high degree of variability, due to their method of formation, which will be described later. Since the description of a second *Melampsorella* species requires authentic telial material, at present lacking, it is not possible to describe this species.

Boyce (3) considers *M. cerastii* to refer to the species on *Picea* sp., *Stellaria* sp., and *Cerastium* sp., whereas the other species is the old *Peridermium coloradense* (Diet.) A. & K. with pycnia and aecia on *Abies* and uredinia and telia unknown.

In the opinion of the writer, there are two species of *Melampsorella* with strikingly different pycnial and aecial morphology but identical in the uredinial and telial stages. Since final proof of this is still lacking, the writer is inclined to follow the temporary arrangement of Boyce. It should be emphasized here, however, that while the description below applies to *M. cerastii*, it is also, in the writer's opinion, true for the species of *Melampsorella* on *Abies*.

The diploid mycelium is perennial and systemic in *Cerastium arvense*. The presence of the mycelium alters the type of growth. Normally, flowering is profuse and continuous (FIG. 1*A*), but the infected plants are sterile or practically so. Infected plants tend to have more branches than normal plants, often having a bunched appearance which is probably a type of witches' broom. This is well shown in the plants in the photograph in figure 1*B*. The infected leaves are considerably reduced in size. The lower leaves are orange on the lower surface, due to the presence of teliospores, but uredinial sori are scattered over both surfaces. The upper leaves bear uredinia only, but the stems are usually sterile. Uredinia may be present also in the floral parts with the result that the flower becomes irregular and often abortive.

After freehand sections had revealed teliospores in various stages of development and germination, fixations were made in Fleming's Weak, Formalin Acetic-alcohol and Navachin's solutions and the material embedded in paraffin, sectioned at 10μ and stained with triple stain.

In the region where this material was collected aecial infections, which result in witches' brooms on both *Abies* and *Picea*, were very numerous. The alternate hosts, *Cerastium* and *Stel-*

FIG. 1, *A*. Photograph of *Cerastium* sp. elev. 10,000 ft. Medicine Bow National Forest, Wyoming. July, 1942. *B*. Close-up of *Cerastium arvense* infected with *Melampsorella cerastii*. The lower leaves are orange on the lower surface due to teliospore formation. Uredosori are scattered over both surfaces of all leaves. Note witches' broom on older plant at right.

laria, are universally present, yet it was difficult to find *Cerastium* or *Stellaria* infected. It required a search of several weeks before sufficient plants were found in order to make fixations. Since the witches' brooms on *Abies* reach a diameter of one to three feet and those on *Picea* up to six feet, the number of aeciospores produced is prodigious; at times red clouds of spores are set free when the broom is disturbed. There are sufficient aeciospores present to inoculate every plant for miles around, yet aecial infections were comparatively rare. Evidently the conditions necessary for the establishment of the systemic mycelium in the crown and underground parts are extremely exacting.

PRIMORDIAL CELLS

The mycelium which ascends vertically in the meristems is also found in the cortex and pith of the stems. In the leaves it grows rapidly through the young tissues, especially in the loosely arranged tissues of the mesophyll. Hyphae are particularly evident in the region lying directly above the lower epidermis, indicating by their presence the region of primordial development. As soon as the leaf tissues are mature and probably earlier, there are indications of primordial cell formation. Hyphae grow to the lower epidermis, branching and spreading through intercellular spaces in close proximity to the epidermal cells. Hyphal cells which are in contact with epidermal cell wall now begin to enlarge. These enlarged hyphal cells become the primordial cells. Their formation is apparently continuous and new primordial cells are added for some time resulting in a layer of hyphal cells, which is very irregular, due to differences in age. Except for occasional breaks these cells form a continuous layer overlying the cells of the lower epidermis.

The primordial cells are highly irregular both in size and shape, depending apparently upon the number of primordial cells and the amount of space available. In spite of this diversity these cells tend to have a shape that is recognizable and to fall within a rather broad size range. They are roughly rectangular or brick-shaped in profile view, and square when seen in a transverse section. From above the primordial cells are seen to be densely packed, ranging from square to slightly oblong or rectangular. Most of the cells

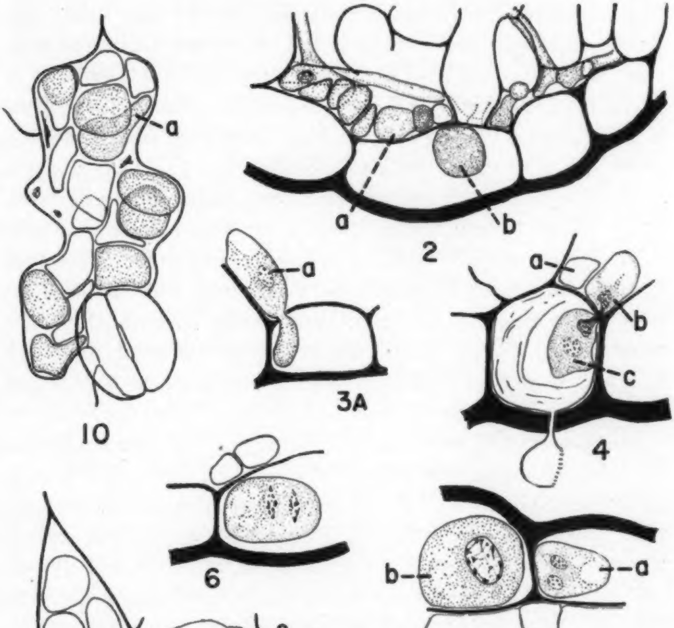
are 10–17μ long and 4–9μ wide. Figure 2 demonstrates the wide variation found in a typical transverse section of this stage with abundant mycelium and young to mature primordial cells, with a typical one at *a*. This figure was drawn from a free-hand section and thus the nuclei are not shown. Typical cells from stained prepared material are shown in figures 3*B*, *a*, *c*; 4*a*, *b*; 9*b*; 11*a*; 16*b*, *c*; 17*e*, and in following figures.

The origin of primordial cells from hyphal cells is indicated in figures 16*c* and 18*e* where the young primordial cell is clearly an enlargement of a hyphal tip. Note that a cell wall has not yet been formed in these figures, whereas in figure 14 the primordial cell (*g*) has been cut off from the hypha (*h*). The irregularity caused by adjacent host cell walls is illustrated in figures 9*b* and 15*a*. The binucleate condition is conspicuous and the dense cytoplasm is finely granular with prominent vacuoles (FIGS. 15*a*, 16*c*).

TELIOSPORE INITIALS

Penetration of the epidermal cell wall is rapidly accomplished and the cytoplasm flows in, forming a slender teliospore initial (FIG. 3*A*) which enlarges rapidly showing conspicuous vacuoles (FIG. 3*B*, *b*). The initial becomes spherical if spatial conditions permit (FIG. 2*b*), otherwise it conforms to the presence of the epidermal cell walls (FIG. 11) and neighboring initials or mature spores (FIG. 9*a*, 15*b*). The nuclei move downward in tandem

FIG. 2. Freehand cross section from fresh material showing mycelium and primordial cells, *a*. and young teliospore initial *b*. 3*A*. Formation of teliospore initial from primordial cell *a*. 3*B*. Typical vacuolate initial with binucleate primordial at *a*, surrounded by other primordial cells, as at *c*. Nuclei preparing to enter initial. 4. Passage of nuclei from primordial cell *b*, to teliospore initial, *c*. 5. Teliospore initial *a*, in upper epidermis, which is uncommon. Note teliospore at *b*, and empty primordial cells below. 6. Young teliospore in which the dikaryon is undergoing simultaneous division to form a two-celled teliospore. 7. Typical unicellular teliospore (*b*) and atypical two-celled teliospore (*a*). Note empty primordial cell *c*. 8. Whole mount of lower epidermis from fresh material showing seventeen young teliospores. 9. Mature binucleate initial *a*, and primordial cell from which it had its origin. Young teliospore with fusion nucleus at *d*, and second layer of teliospores at *e*. 10. Group of mature and germinating teliospores in a single epidermal cell. Whole mount of fresh material. Some teliospores have already germinated and are empty. The spore at *a* is just beginning to germinate.



FIGS. 2–10.

formation (FIG. 14*a*), one nucleus squeezes through (FIG. 14*d*) elongating greatly in the process, and the second nucleus immediately follows (FIG. 4*c*). The remainder of the cytoplasm flows in, providing a binucleate cell with conspicuous vacuoles (FIG. 9*a*) which enlarges rapidly (FIG. 14*c*, *e*). This is the teliospore initial which will give rise directly to the teliospore (FIG. 5*b*) and this term is applied to this cell as long as the binucleate condition obtains. When nuclear division begins, the term teliospore is used.

In *Melampsorella cerastii* the teliospores are single celled (FIG. 8) and arise directly by growth and differentiation of the teliospore initial. Occasionally the initial will divide with small parallel spindles (FIG. 6) and a two-celled teliospore will result (FIG. 7*a*). In a few cases three- and four-celled teliospores have been found, but they are comparatively rare.

It is important to bear in mind that there is a continuous production of initials and new teliospore initials may develop alongside mature teliospores (FIG. 14) or even germinating teliospores (FIG. 15). When new initials develop in cells which now contain only old teliospores, they compress the walls of the latter, since the empty cell walls offer little resistance to the rapidly developing initial (FIG. 16*f*, *g*). With the first crop of initials the epidermal cell may be fairly well filled as in the cell shown in figure 8 which bears thirteen initials or young teliospores. Since this material was from a fresh preparation the nuclear situation could not be determined.

MATURE INTRAEPIDERMAL TELIOSPORES

At first the young uncrowded teliospores are spherical, but as enlargement continues, crowding occurs and the walls, which are thin, are flattened by mutual pressure (FIGS. 10, 15). Typically the teliospores are arranged in single palisade-like layers (FIGS. 8, 11, 13) but in some cases two layers are formed with one teliospore immediately below another (FIGS. 9*d*, *e*, 13*b*). Nuclear fusion was not observed, but it evidently occurs with rapidity since it was extremely difficult to find large binucleate initials (FIG. 9). Since the teliospores germinate without a resting period all that is required for the teliospore to germinate is a single diploid nucleus, and since there is no need for mitotic activity, the teliospores being

unicellular, syngamy apparently occurs as soon as the two nuclei have entered the initial.

The mature teliospores vary in height from 15–24μ and in width from 10–15μ but individual teliospores vary greatly in size since the number of initials and the size of the spores are limiting factors. In small cells a single teliospore may occupy the entire space (FIG. 11*c*) but in larger cells they are naturally more numerous (FIG. 13). There are thirteen in the epidermal cell shown in figure 8, and seventeen in figure 10. The number of teliospores per cell therefore cannot be estimated accurately. In figure 8, for example, the shape of the teliospore indicates a young uncrowded condition, whereas figure 10 shows the development of new initials and crushing of old teliospores which have germinated and are now empty. Usually the majority of the cells of the lower epidermis are filled and it is estimated that 80–90 per cent of the lower epidermis is involved. The fusion nucleus is relatively large and does not enter a resting phase but remains in a characteristic spireme stage, much like the leptonema stage, with delicate but distinct threads (FIGS. 5*b*, 9*c*). This stage could probably be designated as an interphase. As the teliospores enlarge, the epidermal cells become filled and the nucleus increases in size. Figure 11 is typical of the appearance of the teliospores at this state, with dense cytoplasm and a single large nucleus. The nucleolus is not evident here, nor is it ever, in fact, a conspicuous feature of the nuclei of the hyphae (FIG. 14*h*) or primordial cells (FIG. 11*a*).

The lower epidermis of the lower leaves is almost completely involved in the formation of teliospores. In only a few cases are initials (FIG. 5*a*) and teliospores (FIG. 5*b*) formed in the upper epidermis and here the area involved is definitely limited in size and the number of teliospores is correspondingly small.

ATYPICAL TELIOSPORE FORMATION

It is not uncommon to find mature teliospores developing at sites other than the interior of an epidermal cell. The more common situation is where a teliospore develops subepidermally in the intercellular space immediately above the lower epidermis which is already filled with teliospores (FIGS. 11*b*, 16*d*, *e*). The evidence

suggests that the primordial cell became the teliospore *in situ* since these spores are not accompanied by any empty cells of a precursor type. The greatly enlarged primordial cell in figure 11*a* will probably develop in a similar way. Occasionally, groups of teliospores will develop in the middle of a leaf. In a few cases teliospore initials have entered parenchyma cells in the mesophyll and teliospores have developed there (FIGS. 18*a, d*; 29*d*). Guard cells occasionally act as host cells, the enclosed spore conforming closely to the cell wall boundaries.

A rather peculiar situation is recorded in figure 30 which was fairly common in one particular collection. Evidently primordial cells had developed prolifically in the substomatal chambers, which in this case were close together. The result was that several layers of teliospores developed and their rapid expansion had disrupted the underlying stomata. The relationship between hyphal cells, primordial cells, and teliospores is clearly shown here. By simple enlargement some the the hyphal cells have become primordial cells (FIG. 30*d, f, g*); the latter two, however, have entered epidermal cells normally. Nuclear fusion and further enlargement would cause the cells to become teliospores *in situ* (FIG. 30*a, b, c, e*). Whether or not these would germinate normally was not de-

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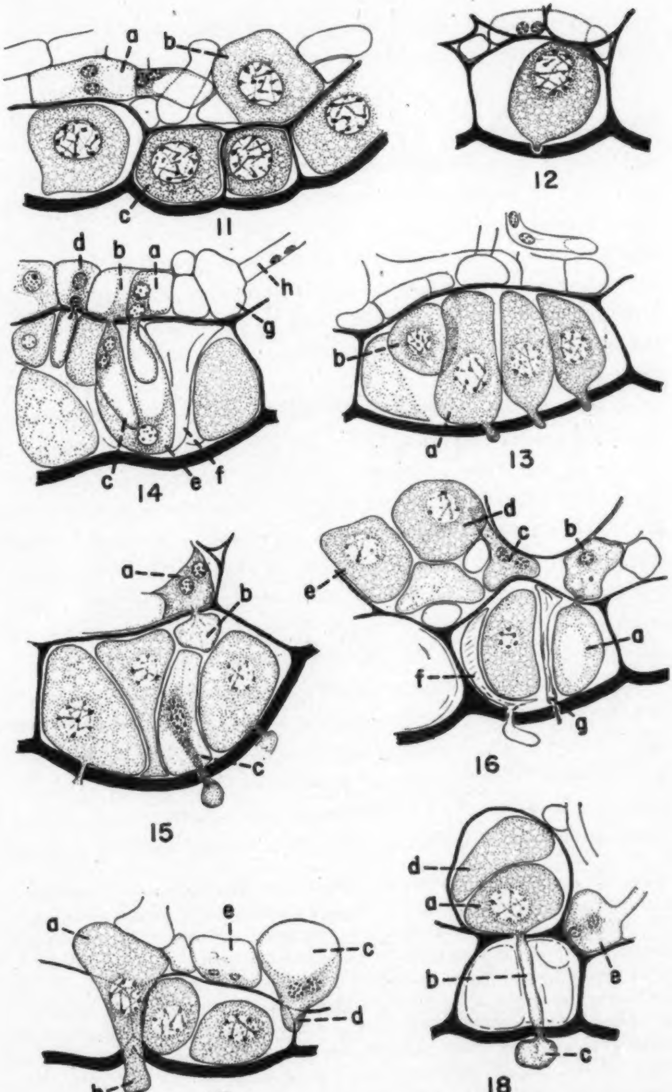
FIG. 11. Group of mature teliospores with characteristic fusion nuclei. The origin of primordial cells (*a*) from hyphae is well shown in this figure. The atypical teliospore (*b*) is in the air space above the lower epidermis. 12. Single teliospore beginning to germinate. 13. Slightly later stage. A later-formed teliospore is shown at a lower level at *b*. 14. Cross section through an epidermal cell to show continuous production of new spores; *a, d*, typical primordial cells with very young initial; *b*, nearly empty primordial cell; and young teliospore initial *c*; *e*, young vacuolate initial; *f*, empty teliospores with crushed walls; *g*, typical primordial cell; *h*, hyphal cell. 15. Group of germinating teliospores, with young initial *b*, developing from primordial cell *a*; *c*, movement of cytoplasm into promycelium. 16. Cross section showing diversity of stages encountered in this material; *c*, very young primordial cell; *b*, mature primordial cell; *a*, young initial; *d, e*, mature subepidermal teliospores; *f, g*, old empty teliospores being crushed by growth of initials and teliospores. 17. Atypical teliospore, *a*, lying in subepidermal space, germinating by forcing promycelium *b*, between the epidermal cell walls. The teliospore initial *c* is sending up a papilla *d*, between the cell walls; *e*, primordial cell. 18. Intramesophyllar teliospores *a, d*. The former has forced a slender germ tube *b*, through the epidermis to form a young promycelium *c*; *e*, young primordial cell.



FIGS. 11–18.

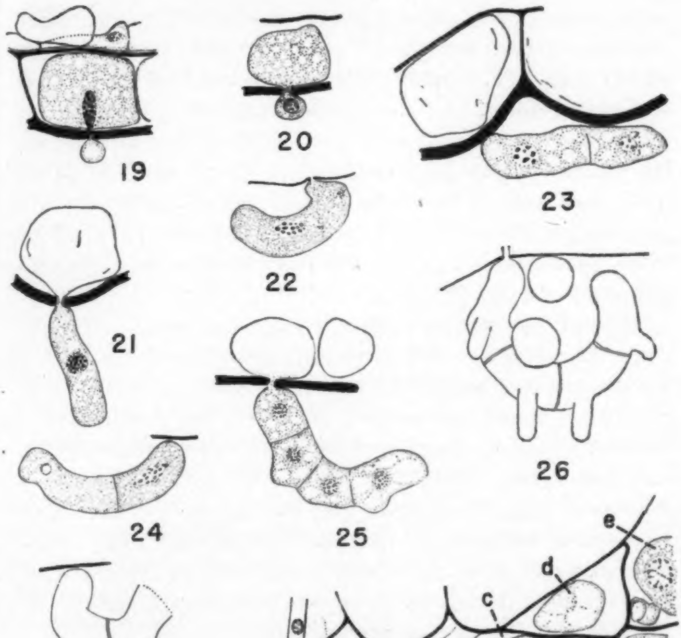
termined. This group of teliospores is suggestive of teliospore formation in the genus *Chrysomyxa*.

GERMINATION

When the teliospore is mature, which would be shortly after nuclear fusion, germination commences. The first evidence is in a small beak or papilla which arises from the spore and projects into the overlying epidermal cell wall. That the invasion is due to chemical rather than mechanical means is suggested by the halo in figure 12 which surrounds the papilla as it begins its passage. The penetration is soon accomplished and an opening is formed which can be identified readily in subsequent stages and through which the papilla emerges (FIG. 13). As the cytoplasm flows outward considerable enlargement of the papilla results and the promycelium comes into being (FIGS. 19, 20). The nucleus decreases greatly in size, the chromatin becoming aggregated into small bodies which have only a faint suggestion of a leptotene stage (FIG. 15*c*). It is possible that this stage represents the actual chromosomes or groups of chromosomes rather than a type of interphase. This appearance is retained as the nucleus which is now greatly reduced in size (FIG. 19), moves downward to the opening, and slips through to occupy the promycelium (FIG. 20).

The cytoplasm sometimes becomes aggregated below the opening in a densely staining band extending from the opening to the nucleus (FIG. 15*c*). In slightly later stages (FIGS. 19, 20) this band is absent, and it is not known whether this is confined to certain cells or if it occurs in all cells but is of short duration. In the ensuing growth period the teliospore is evacuated as the cytoplasm

FIG. 19. Passage of the fusion nucleus into promycelium. 20. Nucleus at base of young promycelium. 21. Fusion nucleus at beginning of meiosis. 22. Meiotic anaphase. Promycelium typically recurved. 23. Two-celled promycelium. 24. Second division in the promycelium. 25. Four-celled promycelium with vacuolate cytoplasm. Sterigmata beginning to form. 26. Typical promycelium from fresh material with sterigmata and basidiospores. 27. Elongated sterigmata, occasionally forked. 28. Mature uninucleate basidiospores. 29. Abnormal germination. The sterigmata of promycelium *b* have crushed the epidermal cell *a*. The promycelium *c* may have arisen from the intramesophyllar teliospore at *d*. 30. Mass of hyphal cells in substomatal chambers forming groups of abnormal teliospores *a, b, c, e; d,* enlarged hyphal cell. Note that even here epidermal cells are being entered in normal fashion from primordial cells *f, g*.



FIGS. 19–30.

moves outward into the enlarging promycelium with the nucleus occupying a central position (FIG. 21). The nucleus enlarges slightly (FIG. 21), forming a delicate network much like that described by Allen (1) for *Puccinia malvacearum*.

The first maturation division immediately follows with the meiotic spindle lying in the central part of the promycelium parallel to the long axis. The spindle is small and spindle fibers inconspicuous; the chromosomes, or groups of chromosomes, are drawn to the poles in an irregular fashion which seems to be typical of this group of fungi (FIG. 22).

Although this material had all stages of germination in abundance, late prophases and particularly metaphases were rare. It was thus not possible to obtain very much evidence as to the haploid and diploid chromosome number. Olive (4) has recently reported the haploid number to be four in the closely-related species *Thekopsora hydrangeae*. Savile (11) has also obtained this number for *Uromyces fabae, U. hyperici; Puccinia sorghi,* and *Melampsora bigelowii,* as well as for *P. malvacearum,* which Allen (1) had earlier considered to be five. In *M. cerastii* the chromosomes were not sufficiently distinct for an accurate analysis, although the anaphase in figure 22 suggests that the number is small.

Two daughter nuclei are organized as the promycelium enlarges and a cell wall is laid down (FIG. 23). The second division follows (FIG. 24) and a four-celled promycelium results. The presence of the promycelia on the surface gives a greyish to pinkish cast to the under surface of the leaf which, up to this time, has retained its orange color. The mature promycelium is typically recurved (FIGS. 25, 26), the amount of curvature showing considerable variation. Even in the young promycelium this curvature is evident (FIGS. 22, 24, 25) suggesting that this structure is negatively geotropic. The nuclei are small but conspicuous and the cytoplasm conspicuously vacuolate (FIG. 25). In size the promycelia measured $31–37 \times 7–9\ \mu$. On the ventral surface of the promycelium small protuberances are formed (FIG. 25) indicating the points of origin of the sterigmata. The sterigmata are elongated and of rather large diameter (FIG. 26), becoming tapered toward the tip (FIG. 27). Figures 26 and 27 were drawn from freehand sections of fresh material and nuclear details were not obtained. The

length of the sterigmata was, on the average, about 7 μ but in certain cases that length was more than doubled. For example in figure 27 the sterigma on the right measured 17 μ, whereas the forked sterigma on the left was 15 μ Savile (11) has pointed out that the length of the sterigmata in certain rusts depends largely upon whether or not there is water on the surface, and this explanation could probably explain the variation that exists here. Certainly the profusion of recurved promycelia on the lower surface would tend to retain a film of water for a considerable period. The passage of the nuclei into the basidiospores was not observed in this material. The basidiospores are round to slightly oblong, measure 7–9 × 6–8 μ, and are uninucleate (FIGS. 26, 27, 28). No evidence has been obtained for further nuclear divisions either in the promycelia or basidiospores.

Allen (1) has shown that the basidiospores of *Puccinia malvacearum* become binucleate before germination. Savile (11) has confirmed this for this species and has shown it to be true also for *Uromyces lespedezae-procumbens* and *Melampsora bigelowii,* and has even reported a quadrinucleate basidiospore. Olive (4) in *Thekopsora hydrangeae,* which is very similar to *M. cerastii* throughout, found that the basidiospore is originally binucleate but one of the nuclei degenerates, restoring the uninucleate condition. Although no evidence of a second division has been found in *M. cerastii* it is not inconceivable that the same condition obtains here as for *T. hydrangeae.*

Occasionally subepidermal teliospores which are lying above the junction of two epidermal cells will attempt to germinate by forcing their way between the cells to the surface. In figure 17, the teliospore, *a,* has already reached the surface and is producing a promycelium, *b.* The teliospore initial *c* is likewise pushing toward the surface at *d.* Teliospores in the cells of the mesophyll may attempt to produce an external promycelium by forcing their way to the surface. In figure 18, two teliospores *a, d,* are in a mesophyll cell, and *a* has produced a long slender germ tube *b,* much like an infection hypha, which has grown completely through the epidermal cell to reach the surface and produce a normal promycelium. Evidently it is necessary for the teliospore to be in close contact with the host cell wall in order to achieve successful

penetration. In the atypical teliospore *e* in figure 29, a slender promycelium has been formed but is held within the boundary of the cell wall. In this same figure portions of two promycelia are drawn at *b* and *c* which were formed above the epidermis in an intercellular space. When the sterigmata were formed, and note that they are produced on the ventral surface, the force of the developing sterigmata crushed the underlying epidermal cell.

DISCUSSION

The development and germination of the teliospores of *Melampsorella cerastii* is in agreement with previous investigations of members of the Pucciniastreae with intraepidermal teliospores. Because of their unusual position and manner of development all of the available information on such forms has been summarized in Table 1.

The most striking features of the table seem to be, first: the general similarity that exists in the nine species that have been described with binucleate primordial cells which are closely applied to the epidermal cell wall and through which entrance is effected with the subsequent formation of a teliospore initial that in most cases divides to produce a multicellular teliospore with thick or thin walls which in turn is highly irregular due to conformity with the confining cell walls and competing spores within the small cell. The second feature is the diversity that exists in the time of formation and also in the time of germination. At one extreme there are the forms which produce the teliospores as soon as the leaves unfold, and proceed immediately to germinate, as *Hyalopsora aspidiotus* and *M. cerastii,* then a second group which produces teliospores during the season, germinating the following spring as *Thekopsora vacciniorum* and *T. hydrangeae,* and a third group in which the teliospores develop on green overwintered leaves, germinating at once, as *Milesia marginalis, M. intermedia,* and *M. fructuosa. Calyptospora goeppertiana* is in a separate group because of the formation of teliospores in the spring when the stems develop, but with germination being delayed until a year later. Of the nine species whose development has been studied only three are alike, *Milesia polypodophila, M. intermedia,* and *M. fructuosa;* all others show a wide variation in kind of spore produced, and in the

TABLE 1

COMPARISON OF SPECIES IN PUCCINIASTREAE WITH INTRAEPIDERMAL TELIOSPORES

	Primordial cells	Type of teliospore	Epidermis		Time of formation	Germination	Author
			Lower	Upper			
Melampsorella cerastii	present	unicellular, thin-walled	present	some	spring, current season	follows immediately	Pady
Hyalopsora aspidiotus	do.	multicellular, thin-walled	present	—	do.	do.	Pady (7)
Thekopsora hydrangeae	absent	multicellular, thick-walled	sometimes	present	fall	on overwintered leaves	Olive (4)
Thekopsora vacciniorum	present	multicellular, thin-walled	present	few	do.	do.	Pady (5)
Milesia marginalis	do.	do.	do.	some	following spring on overwintered leaves	follows immediately	Pady (5, 6)
Milesia polypodophila	do.	do.	do.	—	do.	do.	Pady (5)
Milesia intermedia	do.	do.	do.	—	do.	do.	Pady (5)
Milesia fructuosa	do.	do.	do.	few	fall	on overwintered leaves	Pady (5)
Calyptospora goeppertiana	do.	multicellular, thick-walled	in epidermis of stems		spring	on overwintered stems	Pady (5)

time when the teliospores are formed, but are particularly diversified as to the time when germination takes place (Table 1).

It will be noted that *Hyalopsora aspidiotus* and *M. cerastii* are very similar. The mycelium in both cases is systemic and diploid; the teliospores develop in the lower epidermis of the young leaves as they open in the spring; the teliospores are thin walled and germination follows without a resting period. They are unlike only in the number of cells found in the mature teliospore. This would indicate a fairly close relationship which is still further strengthened by the fact that aecial hosts are the closely related genera *Abies* and *Picea* (Table 2).

In Table 2 a summary has been made of the species of rusts with intraepidermal teliospores in order to emphasize the close relationship that exists among the hosts. For the sake of completeness the second species of *Melampsorella,* as yet unnamed, has been included. It is of interest to note that for all ten species the aecial hosts are confined to three genera of the Gymnospermae and two of these, *Picea* and *Abies,* are closely related. Moreover, seven of the ten species are found on *Abies,* two are on *Tsuga,* and the remaining one on *Picea.* Even among the telial hosts only five families are present, five species going to fern genera in the Polypodiaceae, the remainder going to members of the Caryophyllaceae, Vacciniaceae, Ericaceae, and Hydrangeaceae in the Angiospermae.

Perennial mycelium especially in the diploid phase is unusual, yet in seven cases there is a perennial mycelium; in *Melampsorella* in the aecial and telial hosts; in *Hyalopsora* in the telial host; *Milesia polypodophila* in the aecial host and *Calyptospora* in the telial host. The tendency is for this perennial mycelium to form witches' brooms, particularly where the perennial mycelium is systemic. The one exception to this is *Hyalopsora aspidiotus* which does not show any witches' broom effect. This is doubtless due to the fact that the telial host is a fern, *Phegopteris,* which lacks permanent aboveground parts, dying back each fall to the underground rhizome. The most conspicuous witches' brooms are in the genus *Melampsorella* in which we have witches' brooms on both hosts, those on *Picea* and *Abies* growing for many years and often reaching a remarkable size (**10**).

Olive (**4**) has recently described the development of the spore

TABLE 2

HOST RELATIONSHIPS OF THE SPECIES OF PUCCINIASTREAE WITH INTRAEPIDERMAL TELIOSPORES

Rust species	Aecial host(s)	Haploid mycelium	Witches' brooms	Telial host(s)	Diploid mycelium	Witches' brooms
Melampsorella cerastii	*Abies* sp.	perennial, locally systemic	conspicuous, up to 3 feet diameter	*Cerastium* sp., *Stellaria* sp.	systemic perennial	diffuse, small
Melampsorella sp. (*Peridermium coloradense*)	*Picea* sp.	do.	very large, up to 6 feet diameter	*Cerastium* sp., *Stellaria* sp.	systemic perennial	diffuse, small
Hyalopsora aspidiotus	*Abies balsamea*	grows 2 yrs.	—	*Phegopteris dryopteris*	systemic perennial	—
Thekopsora hydrangeae	*Tsuga canadensis*, *Tsuga caroliniana*	—	—	*Hydrangea arborescens*, *H. radiata*, *H. cinerea*	overwinters	—
Thekopsora vacciniorum	*Tsuga canadensis*	—	—	*Vaccinium* sp., *Azalea* sp., and other genera	overwinters	—
Milesia polypodophila	*Abies balsamea*	perennial	loose type	*Polypodium virginianum*	overwinters	—
Milesia marginalis	*Abies balsamea*	—	—	*Dryopteris marginalis*	overwinters	—
Milesia intermedia	*Abies balsamea*	—	—	*Dryopteris intermedia*	overwinters	—
Milesia fructuosa	*Abies balsamea*	—	—	*Dryopteris spinulosa*, *D. americana*	—	—
Calyptospora goeppertiana	*Abies* sp.	—	—	*Vaccinium* sp.	systemic perennial	large

forms in the long cycled rust *Thekopsora hydrangeae*. He was able to follow the development of the teliospores as they matured at the end of the growing season and their subsequent germination the following spring. Although his account follows the same general pattern of development as found in other species with intraepidermal teliospores (Table 1), it is most similar to that of *T. vacciniorum* which has been previously described (**5**) but from which it differs in several respects. In the first place, primordial cells are not specifically described by Olive but his figures show clearly the presence of similar structures. Since the teliospores are in the upper epidermis the absence of intercellular spaces results in slender vertical hyphae from which the teliospores arise. A similar situation was described for *T. vacciniorum* (**5**) with the difference that in the intercellular spaces above the lower epidermis typical primordial cells were produced. In figure 130 in Olive's paper a teliospore is shown in the lower epidermis, but immediately above is a group of empty hyphal cells which are similar to the primordial cells described in this paper. Intramesophyllar teliospores also occur in *T. vacciniorum* and Olive has shown them also to be present in *T. hydrangeae*. His figures 131 and 132 show spores in the palisade cells, whereas the atypical teliospores found in the outer region of a uredinial sorus might be compared with the teliospores in the substomatal hyphal complex in *Melampsorella* (FIG. 30).

An interesting characteristic described by Olive (**4**) is the simultaneous passage of the nuclei from the primordial cells into the teliospore initials, the nuclei going through as a very attenuated team, whereas in *Melampsorella* the nuclei pass through in typical tandem fashion (FIGS. 4, 14*d*).

An unusual feature of teliospore development in *M. cerastii* is that several crops are produced in successive waves during the early part of the growing season. Most rusts produce a single crop of teliospores as the host approaches maturity, example *Puccinnia graminis*. In the group with intraepidermal teliospores *Thekopsora vacciniorum, T. hydrangeae, Milesia fructuosa* are "normal" in this respect at least (Table 1). Even in the closely related species *Hyalopsora aspidiotus* only one group of teliospores develops and germinates. The growing habits of the hosts may

partially explain this difference. The host of *H. aspidiotus, Phegopteris dryopteris,* is a fern with a small frond which unfolds at one time, allowing the systemic mycelium to invade the entire leaf rather uniformly. *Cerastium arvense,* the host of *M. cerastii,* is a member of the Caryophyllaceae with indeterminate growth, the lower leaves being the oldest with the youngest leaves nearest the tip, except where axillary growth has provided new leaves. The lower leaves of the plant therefore are invaded first and the mycelium is soon well established and teliospore formation follows immediately, which is indicated by deep orange color of the lower surface. Germination begins first in these lower leaves since these leaves and their enclosed teliospores are the oldest. It is at this point that the difference occurs. As the host cells become emptied through the process of germination new teliospores are continually added (FIGS. 5, 14–16) and apparently this process continues over a period of several weeks. *M. cerastii* thus differs from other related forms in having an extended period of continuous teliospore production.

In previous papers (**6**, **7**) attention was called to the peculiar nuclear situation which was found in the mature spores, namely, that instead of being in a resting condition, the chromatin of the mature teliospore was in prophase with a distinct spireme. This is not difficult to understand in those forms like *Hyalopsora aspidiotus* where germination follows immediately, but in a species like *Milesia marginalis* where a resting period followed it was rather surprising. In *M. cerastii* the mature fusion nucleus has never been found other than in a well-defined spireme (FIG. 11).

Olive (**4**) is not clear as to the stage in which the teliospore nucleus passes the winter, although his figures 125–128 show details of the fusion nucleus which is in the spireme stage.

From the cytological standpoint *Melampsorella cerastii* is very similar to the other rusts listed in Table 2, particularly with reference to the details of fusion and meiosis in the promycelia. In one respect, however, it was unusual, namely, in the absence of a nucleolus. The material fixed in Fleming's Weak and F. A. A. was carefully examined for this structure. In other rusts, such as *Hyalopsora* and *Milesia,* the nucleolus is conspicuous up to and including the early stages of nuclear fusion. Moreover, with Flem-

ing's fixatives and the triple stain the nucleolus stands out brilliantly. In this material, however, careful search has not revealed its presence.

According to Savile, the so-called nucleolus of the rusts is not a true nucleolus but an endosphere surrounded by an ectosphere, the true nucleolus being described as a small granule within the endosphere. Rust nuclei exist in two phases, the large expanded type with definite endosphere (nucleolus) surrounded by the ectosphere which is often hyaline, and the small unexpanded type which consists solely of the endosphere. The latter type is found throughout most of the life cycle and is associated with passage through a very small pore. The expanded form originates from the endosphere by the formation of an outer ectosphere into which all of the chromatin passes. In reverting back to the unexpanded form, the endosphere is discharged and the ectosphere reforms into a small nucleus about the size of the original endosphere. Endospheres were not found in *M. cerastii* and it is not possible with this material to confirm this endosphere hypothesis. That the two types of nuclei probably do exist in *M. cerastii* is suggested from a study of the nuclear sizes which show both small unexpanded and large expanded nuclei, but an endosphere as such is completely lacking. The presence of a so-called true nucleolus in the form of a granule also could not be confirmed.

SUMMARY

The teliospores of *Melampsorella cerastii* are developed in the lower epidermis of the young leaves of *Cerastium arvense* in the spring from a systemic perennial mycelium. Hyphae mass up above the cells of the lower epidermis and from certain hyphal cells of the fungus enlarged binucleate primordial cells are formed. Each primordial cell penetrates the host cell wall and the contents flow in to form a teliospore initial which, by growth and differentiation, develops directly into a single-celled thin-walled teliospore.

Following nuclear fusion the teliospore immediately germinates, producing a slender recurved promycelium into which the fusion nucleus migrates. Meiosis occurs here and four uninucleate basidiospores are produced.

Teliospores completely fill all of the cells of the lower epidermis of the lower leaves, giving the leaf an orange color. Some telio-

spores are formed in the middle leaves but are lacking in the upper leaves. Teliospore formation is continuous during the first few weeks and new initials are formed in the cells as the teliospores germinate.

Comparisons were made with the other rust species with intra-epidermal teliospores which have been examined cytologically. *Melampsorella cerastii* is similar in its general development but differs principally in that the teliospore is unicellular.

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EXPLANATION OF FIGURES

Note: All figures are drawn with camera lucida and are oriented in their natural position, that is, with the lower epidermis facing the bottom of the page. Figures 1, 8, 10 × 513, figures 2–7, 9 × 1153, all other figures × 990.

THE TAXONOMIC POSITION OF PHOLIOTA MUTABILIS AND RELATED SPECIES

Rolf Singer and Alexander H. Smith *

(with 10 figures)

It has been recognized for a long time that the genus *Pholiota* in the Friesian sense is artificial. The characters of the veil were greatly overemphasized by Fries and even more so by Schroeter and others. In Fries' arrangement *Rozites caperata* was placed in the same genus as *Pholiota squarrosa* simply because the partial veil, when it breaks, leaves an annulus on the stipe. It is as logical to insist that these two species be in the same genus because of this one similarity as it is to insist that certain species of *Lepiota* be excluded from that genus simply because the veil tissue is delicate and does not remain on the stipe in the form of a membranous ring when the veil breaks. In any practical classification these Lepiotae are grouped together because of their obvious affinities with species possessing truly membranous rings. The dark spored agarics, in particular *Psathyrella* emend. Kühner, furnish another excellent example of a very homogeneous series of fungi in which the disposition of the veil remnants on the stipe is not important at the generic level. In *Psathyrella* it appears logical, in order to arrive at a natural and usable classification, to group together parts of several of the Friesian genera which lack fundamentally distinctive characters. The fragile, annulate Strophariae, for instance, are now placed in *Psathyrella*. However, in *Pholiota* the opposite situation has prevailed. Here it is necessary to divide the genus into several groups at the generic level if one is to arrive at the most practical and natural grouping of the various species. Some of the groups already segregated from *Pholiota* by others are:

* Papers from the Farlow Herbarium, Harvard University, Cambridge, Mass., and Paper No. 832 of the Dept. of Botany and University Herbarium, University of Michigan, Ann Arbor, Michigan.

Agrocybe Fayod (1889), *Gymnopilus* Karsten (in the sense of *Fulvidula* Romagnesi), *Rozites* Karsten, and *Pholiotina* Fayod. This has left two groups of species in what might be regarded as *Pholiota* sensu stricto. One of these groups is relatively large and is typified by *P. squarrosa* (Muell. ex Fr.) Quél., *P. squarrosoides* Pk., etc. The best known representative of the second is *P. mutabilis* (Schaeff. ex Fr.) Quél. It differs from species in the first group in having spores with a distinct germ pore at the apex much as in species of *Psilocybe,* and in having the epicutis of the pileus composed of narrow appressed hyphae. In addition the pilei are hygrophanous and naked, and frequently have translucent striae on the margin caused by the gills showing through the thin, moist flesh. However, *P. mutabilis* has never been separated from *Pholiota,* no doubt because it was the only species known to possess the above characters and because the squarrose scales of the stipe made it easily included in the generic description of *Pholiota* sensu stricto.

However, these are not valid reasons for continuing to include *P. mutabilis* in *Pholiota.* Our study has brought to light four species which have practically all the important anatomical and macroscopic characters in common with *P. mutabilis.* In two of these the veil is too poorly developed to leave scales on the stipe. One has scales which are smaller and more indistinct than those of *P. mutabilis,* and which are usually confined to a narrower zone beneath the annulus. In all these species we have noticed a slight viscidity caused by the subgelatinous nature of the cuticle. The layer, however, is not thick enough or sufficiently gelatinous to render the caps distinctly viscid under all conditions. All four species have small, thin, hyaline cheilocystidia and small ovoid spores characterized by a broad germ pore. The known species are all lignicolous and typically vernal in their fruiting habits. Later fruiting periods are known for most [1] but our information indicates that their peak of fruiting-body production is reached during the spring.

It is interesting to note that these characters are also found in most species of a group of dark spored agarics which some authors

[1] In a species found in Florida collected in July 1943 there is not enough data available to indicate a seasonal fruiting pattern.

recognize as the genus *Deconica*.[2] When one considers the problem of the possible relationship of these two groups he is at once impressed by their similarity in all fundamental characters except the color of the spore deposit, and a survey of the literature causes one to question whether the color of the spore deposit in the *Pholiota-Flammula-Naematoloma-Psilocybe* (*-Deconica*)-series of species is really significant. Smith in 1943 described two specices of *Naematoloma*[3] under the name *Hypholoma* in which the spores were typically dull brown in deposits. It has long been known that *Naematoloma elongatum* has a typically brown spore deposit (it has been described as a *Naucoria*). In addition, a number of species which have been described in *Naucoria* have been found to belong in *Psilocybe* (*Deconica*) or in *Naematoloma*. This clearly indicates that color of the spore deposit is not a sufficiently distinct character for grouping species into genera in those fungi most closely related to *Pholiota mutabilis*. In view of the striking similarities of the morphological and anatomical characters of the two groups this situation leads us to regard them as very closely related and brings up the question of the relationships of *Pholiota* sensu stricto, *Flammula* sensu stricto, *Stropharia, Neamatoloma* and *Psilocybe* (including *Deconica*). These genera need to be restudied critically with an eye to reëvaluating the use of the annulus as a generic character for separating *Pholiota* and *Flammula* on the one hand and *Stropharia* from *Naematoloma* and *Psilocybe* on the other. We are by no means the first to see the need for this study —it has been very ably pointed out by Kühner (**1936**). We strongly doubt whether it is desirable to continue to maintain both *Pholiota* and *Flammula* as separate genera. The only difference between them as defined here is in the degree to which the veil is developed. The number of species in which the veil is "subannular" is greater than either of the extremes. In a number of species the presence of an annulus is a variable character, as in *Pholiota malicola*. In the purple brown spored series, however, it appears desirable to recognize both *Naematoloma* and *Stropharia*.

[2] The senior anthor recognizes *Psilocybe* and *Deconica* as separate genera wheras the junior author places them in one genus, *Psilocybe*.

[3] **Naematoloma olympianum** Smith. comb. nov. (*Hypholoma olympianum* Smith, Mycologia **36**: 248 (1934) and **N. subochraceum** Smith, comb nov. (*Hypholoma subochraceum* Smith, *Ibid.* p. 251).

Returning to a consideration of the similarities and possible relationships of the rusty brown and dark-spored groups of genera previously mentioned, we can go one step farther and consider a group of species which Patouillard named *Melanotus*. These have fruiting bodies resembling those of *Crepidotus*, but the species are actually pleurotoid *Deconicae*. We accept this genus. Parallel to it the senior author has found an unnamed species confused with *Crepidotus* by some authors. It has thick-walled, smooth spores and falls in this group, but is more closely related to the Pholiotoideae of the senior author's classification. We do not mean to suggest that spore color is of no value as a character in this particular group, and we do not recommend that parallel genera separated mainly by spore color be united into large genera. But it does appear that after consideration of the very striking parallelism in both series, and the presence of intergrading species, the subfamilies Pholiotoideae as defined by the senior author in 1936 as a subdivision of the Cortinariaceae should be combined with the Stropharioideae as defined by Romagnesi and the senior author as a subdivision of the family Coprinaceae into a single independent family. This manner of viewing the affinities among these major divisions of the Agaricales is not entirely new; in fact it has been expressed by Konrad & Maublanc (**1924–37**) who referred the genus *Naematoloma* to the tribus Pholiotées (containing among others such genera as *Flammula* and *Pholiota*). The senior author also expressed it by referring *Melanotus* (**1936**: 343) temporarily to to the Cortinariaceae, Pholiotoideae. In contrast with the earlier views of the senior author and with the classification of Konrad & Maublanc we would now exclude the genera *Rozites* (near *Hebeloma* of the Cortinariaceae sensu str.) and *Crepidotus* (near *Ripartites,* Paxillaceae sensu str.) from the Pholiotoideae. We would also exclude *Phaeolepiota* which Heim and the junior author believe to be related to *Cystoderma*. For this family we propose the name Strophariaceae.

Strophariaceae fam. nov. Pileo viscido vel subviscido-opimo vel subudo, hygrophano vel non hygrophano; cuticula ex epicute et hypodermio consistente, epicute ex hyphis filamentosis haud regulariter palisadiformiter nec hymeniformiter dispositis; hypodermio saepissime ex hyphis latiusculis vel breviusculis, interdum parietibus crassiusculis praeditis consistente; velo annuliformi, marginali, fibrilloso atque fugaci, vel subnullo; sporis in cumulo

atrolilacinis vel atrofuscis aut ferrugineo-cinnamomeis; sporis membrana duplici, levissima, apice interrupta vel attenuata poro germinativo angusto, indistincto vel lato truncato causa; lamellis nebulosis, varie adnexis (subliberis vel decurrentibus); cheilocystidiis constanter praesentibus; cystidiis prope aciem interdum differentiatis, vel pleurocystidiis refringentibus distinctis praesentibus, vel nullis; stipite variabilissimo, interdum laterali minutissimoque; carne molli, in stipite fibrosa, pigmento intercellulari saepissime praesente, interdum amara; mycelio interdum rhizomorphoideo albo, lignicola, terricola, fimicola, herbicola, muscicola, etc. Genus typicum *Stropharia* (Fr.) Quél. Genera cetera: (Amaurospora): *Naematoloma* Karst., *Psilocybe* (Fr.) Quél., *Deconica* (W. Smith) Karst. *Melanotus* Pat. (Ochrospora): *Pholiota* (Fr.) Quél., *Flammula* (Fr.) Quél. *Kuehneromyces* Singer & Smith, *Pleuroflammula* Singer.

Kuehneromyces gen. nov. Pileo glabro (vel fibrillis inconspicuis e velo nascentibus ad marginem ipsum ornato), nudo opimo-viscidulo, hygrophano, marginem versus pellucide striato in humidis, cinnamomeo-brunnescente; epicute ex hyphis subparallelis, tenuibus, hyalinis, subgelatinescentibus, jacentibus, fibuligeris efformata; subcute ex hyphis irregularibus, latiusculis, demum saepissime crassotunicatis efformata; dermatocystidiis nullis; lamellis cheilocystidiis sparsis vel numerosis ad ipsam aciem concentratis et interdum cheilocystidiis prope aciem congregatis praeditis; cystidiis aliis (typi *Flammularum*) nunquam ullis; tramate regulari, ex hyphis subintertextis vel intertextis fibuligeris hyalinis vel brunnescentibus formato; sporis membrana duplici, levissima instructis, minutis, ovoideis vel ellipsoideis, ad apicem poro lato germinativo truncatis, haud vel vix lentiformibus, melleis, in massa cinnamomeis vel brunneis (neque umbrinofuscis nec purpurascente-fuscis nec obscure lilaceis); stipite plerumque centrali, elongatoque, farcto dein cavo, velato, squarroso vel nudo, annulato vel veli reliquiis fibrilloso vel subevelato. Ad ligna, fructificationibus praecocibus. Species typica: *K. mutabilis* (Schaeff. ex Fr.) Singer & Smith (*Pholiota mutabilis* auct.). Species ceterae adhuc cognitae: *K. rostratus* Singer & Smith; *K. depauperatus* Singer & Smith; *K. vernalis* (Peck) Singer & Smith.

We name this genus for Robert Kühner who was the first author to point out that *K. mutabilis* is not a true *Pholiota*. He says (**1935**: 31, footnote 2) "Nous faisons, peut-être nous-même, preuve de timidité en laissant provisoirement dans le *Pholiota*, le *Ph. mutabilis;* cette espèce semble assez peu éloignée du *Ph. marginata*, mais ses spores lisses à pore germinatif tronqué l'éloignent de tous les *Galerina* que nous connaissons."

KEY TO SPECIES

A. Typically northern species, growing cespitosely (sometimes solitary); color of dried pilei 9, G5 or darker ocher brown.
  B. Stipe with numerous distinct recurved scales in the portion beneath the annulus when young and fresh, darkening from the base upward in age; cheilocystidia small (19–29 × 3.3–7 μ) and gill edge not heteromorphous .... *K. mutabilis*, no. 1.

B. Stipe not at the same time squarrose and darkening from the base upward; cheilocystidia large or small, sometimes of two different types.
  C. Cheilocystidia with long, thin neck, seldom nodulose; stipe 4–12 mm. broad and not appreciably darkening with age
  K. rostratus, no. 2.
  C. Cheilocystidia often with short or thick neck, which in a large number is nodulose at the tip, two distinct types sometimes present; stipe 2–8 mm. broad, becoming russet to mummy brown from the base upward in age............................*K. vernalis*, no. 4.

A. Typically southern species (Florida), with solitary habit (as far as known); color of dried pilei 9, F5 to 10, F5 (Maerz & Paul), i.e. with a more reddish tinge than in the northern forms...*K. depauperatus*, no. 3.

DESCRIPTION OF SPECIES

1. **Kuehneromyces mutabilis** (Schaeffer ex Fr.) comb. nov. Figures 2–3 & 8.

Agaricus mutabilis Schaeff. ex Fr. Syst. Myc. **1**: 245. 1821 (var. *b, c, d* exclusis).

Pholiota mutabilis Quélet, Champ. Jura et Vosges p. 94. 1872.

Dryophila mutabilis Quélet, Enchir. Fung. p. 69. 1886.

Pileus 15–60 mm. broad, obtuse when young (rarely papillate), becoming campanulate or broadly conic while the margin is still strongly incurved, expanding to convex or plane or retaining a low broad abrupt umbo, the margin often remaining decurved, surface glabrous or with inconspicuous white fibrils from the veil when very young, smooth, lubricous to viscid from a more or less separable pellicle (merely moist after heavy rains have washed off the pellicle), margin closely translucent striate when moist, opaque when faded, hygrophanous, "clay color" to "ochraceous tawny" or "sayal brown" at maturity, near "Verona brown" when young, fading to near "pinkish buff" or more nearly "ochraceous buff" on disc, fading from the disc first or in a zone between disc and margin; flesh thin except in the disc, moderately soft, watery to moist, pallid, odor weak, agreeably spicy (neither radishlike nor farinaceous), taste mild or slightly unpleasant but not bitter; lamellae close to crowded (about 35 reach the stipe), broadly adnate to subdecurrent when young, later usually distinctly decurrent (more so than in other species), broad in inner third (about 5 mm. in medium sized mature caps), pallid when young, developing a dull buff tinge and eventually becoming almost "Sayal brown"; stipe 40–100 × 2–12 mm., subequal to evenly tapering toward the base, stuffed and soon becoming hollow, with an apical to subapical annulus or annular zone of fibrils, below the annulus covered almost

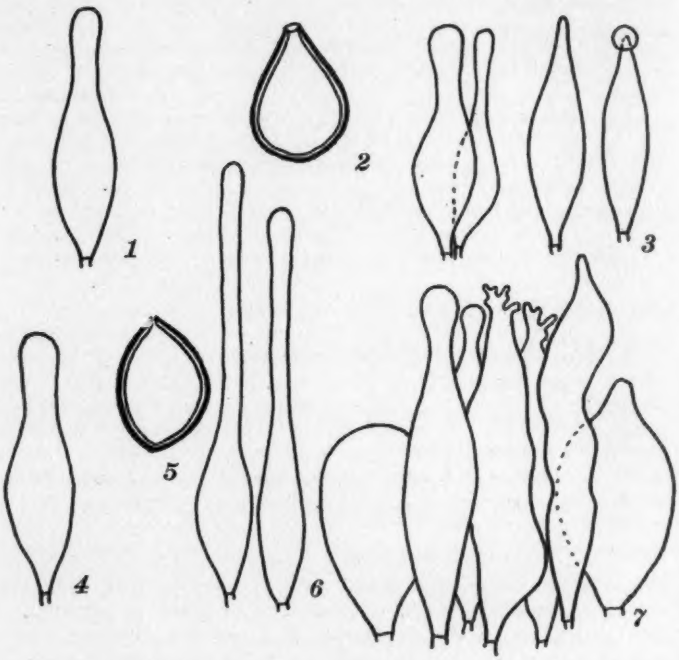

FIG. 1. *Kuehneromyces depauperatus,* a cheilocystidium from the type collection. FIGS. 2–3. *K. mutabilis;* 2, spore in approximately frontal view; the outer black line showing the exosporium, the white zone between the outer black line and the inner (thin) black line representing the endosporium. Note the broad germ pore at the apex; 3, four cheilocystidia. The two at the left represent the most common type (from American material). The central figure is from European material and represents a rare type. At the right is a cystidium from Javanese material showing the drop of mucilage at the apex. FIGS. 4–5. *Pleuroflammula Dussii;* 4, cheilocystidium (from type collection); 5, spore. The outer black line represents the exosporium, the inner black line the bright colored ring inside the endosporium; the white zone between the two black lines the endosporium. Note the narrow germ pore at the apex (from the type). FIG. 6. *Kuehneromyces rostratus,* two cheilocystidia. FIG. 7. *K. vernalis.* Five cheilocystidia in the middle representing type I; these flanked by cheilocystidia of type II (from material collected in Wyoming).

to base with distinct pallid to brownish recurved scales, lower surface of annulus also scaly in some specimens, scales sometimes indistinct in dried material, base either naked or covered by a white velutinous mycelial tomentum, somewhat silky-striate above annulus, pallid at first over all except basal portion, soon becoming brownish (near "clay color"), finally becoming dark sordid brown from base upward ("Dresden brown" to "mummy brown").

Spore print between "Verona brown" and "cinnamon" (R) or between 176 and 191 (Séguy) when quite fresh, spores under a microscope deep honey color or more fulvous-castaneous in accumulations, 6–7.5 (rarely 11) × 3.7–5.8 (6.2) μ, smooth, ovoid, the hilar end very broadly rounded, the broadest portion near the base, in a minority more ellipsoid with thickest portion near middle, rarely somewhat subrhomboid, terete as seen in end view or up to 0.6 μ broader in front than in side view, not distinctly compressed, as seen in side view less convex on inner side, without a suprahilar depression, with a double rather thick wall and a very broad flat germ pore (hence truncate); basidia four-spored; cheilocystidia rather uniform in shape and size, abundant but gill edge not heteromorphic, 19–29 × 3.3–7 μ, hyaline, ventricose to more rarely subfilamentous below, thickest in the middle or slightly below it, apex ampullaceous with a cylindric or very slightly subcapitate tip and neck cylindric, with an exudation that in KOH often enlarges to a globose appendage-like body reminiscent of the capitate cheilocystidia of many species of *Conocybe*, neck 6–11 × 1.8–4 μ; pleurocystidia not differentiated; gill trama regular, the hyphae somewhat interwoven, with brownish incrusting pigment on the walls (more pronounced in age); pileus trama of interwoven irregular hyaline hyphae which in old carpophores have somewhat thickened walls; hypoderm of similar hyphae but with heavier incrustations of pigment; epicutis consisting of closely appressed narrow filamentous subparallel hyphae forming a rather conspicuous layer at first but gelatinizing and eventually almost disappearing, all hyphae with clamp connections, non-amyloid.

The fruiting bodies occur cespitosely or densely gregarious-subcespitose, rarely isolated, and they often literally cover the stump or log upon which they grow. More rarely they are found on buried wood, on decaying boards or on beams, and prefer *Fagus, Populus, Betula, Alnus, Quercus,* etc., but occur even on *Rubus,* and more rarely on conifers. The fruiting begins in April and continues until December, depending on the region and precipitation.

MATERIAL STUDIED: **Nova Scotia,** Colchester Co., on *Betula lutea* in August, *A. H. Smith* (*Wehmeyer, 783*), (MICH.). **New York,** Adirondack Mts., September, *C. H. Kauffman* ("Rare in this country. The first collection I know of"), (MICH.). **North Carolina** and **Tennessee,** Great Smoky Mts. National Park, Mt. Leconte, *A. H. Smith 10478* (MICH.);

FIG. 8. *Kuehneromyces mutabilis.* × 1.

Grassy Patch, on *Betula,* June, *L. R. Hesler, 12536* (FH, MICH.); Roaring Fork, *A. J. Sharp 107* (FH); Indian Gap, on *Betula,* June, *L. R. Hesler 11467* (FH), *13967* (MICH.); *L. R. Hesler* and *S. L. Meyer 12701* (MICH.). **Colorado,** Gunnison River, among willows, August, *E. Bartholomew 2655* (FH, determination doubtful). **Washington,** on wood of *Alnus rubra* and *Populus trichocarpa,* many collections between May and October,

A. H. Smith, 13426, 13709, 13741, 13620, 13918, 14265, 14534, 14721, 16163, 16252 (MICH.); Lake Quinault, on *Rubus parviflorus,* October 14, 1925, *C. H. Kauffman* (MICH.). **Oregon,** Rhododendron, on *Alnus,* October, *Gruber* and *Smith 19540* (MICH.). **Europe:** The senior author has collected fresh material at many stations in the U.S.S.R., Czechoslovakia, Austria, Germany, France, Switzerland, Italy, and Spain; material is preserved in the European herbaria covering nearly every region of that continent; good material is preserved in the Hoehnel Herbarium (FH) from Austria, Niederoesterreich, Schneeberggebiet, also from the Wiener Wald, and from Aspang, etc.; also in the Burt Herbarium (FH), from Sweden, Ekero, *L. Romell,* and Uppsala, *E. A. Burt;* also in the Bucholtz Herbarium, from Russia, *44* (FH), and in the University of Michigan Herbarium from France, Humont near Plombière, Vosges, *M. Josserand;* this material was restudied and found to be identical with the American collections. **Central Asia:** Altai Mts., Oirotia, *R. Singer* and *L. N. Vassilieva* (LE). **Caucasus Mts.,** Mount Oshten in *Fagus-Abies* woods, *L. N. Vassilieva* (Herb. Cauc. Nat. Res.); Guzeripl, on dead stump of *Abies Nordmanniana,* and on stump of *Fagus orientalis, L. N. Vassilieva* (Herb. Cauc. Res.); Saken River valley on *Abies* trunk near timber line, and Umyrka River Valley, on stump of *Betula,* near the timber line, also Alous, in fir woods, *L. N. Vassilieva* (LE); Nenskryra Valley, Saken Valley, Khodsha Range, and Klytch Valley, *R. Singer* (W); Saken, twice on conifers, once on root of *Corylus* sp., *R. Singer* (W). **Java,** Tjibodas, *F. v. Hoehnel* (FH).

The most remarkable locations are those in the high mountains, near the timber line on conifers, and in the tropics in Java. Most authors indicate only frondose wood for this species, but there is no doubt that, especially in the mountains, it grows on conifer wood also. The senior author published on its occurrence on the wood of *Pinus mugho* in the Alps (Jägerkamp near Schliersee, Bavaria), in June at about 1600 m. elevation, i.e., near the timber line. This material had aberrant spores ($9–10 \times 5–6\,\mu$), see Zeitschr. f. Pilzk. **4**: 40, no. 48, 1925. We have not been able to check on the basidia, but it is reasonable to assume that we are here dealing with a two-spored or mixed form such as we have observed in *K. vernalis,* a condition often found in the subalpine and alpine zones. However, the substratum is not correlated with spore size. Other collections on *Abies* from lower elevations had typical spores. It appears likely that here we have another example of the rule indicated by Heim and the senior author (Rev. de Myc. **1**: 76. 1936) regarding the relation between spore size and altitude.

The Javanese material is abundant and in good condition. There is no doubt but that it is typical *K. mutabilis* although some speci-

mens are remarkably small. Some are of normal size, however, and we find specimens in America, from the Great Smoky Mountains for instance, which are typically smaller than those from the west and north. The locality within the Tjibodas Forest Reserve was not indicated by Hoehnel, but it may well be that these specimens were collected at a high elevation, in the "cool" or "cold" zone on Mt. Gedeh where tropical-alpine vegetation predominates, with many plants closely related to temperate and northern types. It should be remembered that there are several species of oak almost everywhere in that forest, and these would make logical substrata for this fungus.

Kauffman thought his collection on *Rubus* was a distinct variety of the species. After what we have seen regarding the kinds of wood *K. mutabilis* can use as a substratum, this one, even though unusual, is not altogether surprising. A careful study of the specimens failed to reveal any further distinguishing character other than the one Kauffman had emphasized, *i.e.*, the viscid pileus. Since the viscidity in *K. mutabilis* is variable depending on the locality, the weather, and age of the fruiting body, we cannot attach any importance to it here.

K. mutabilis, in our opinion, is the most primitive species of the genus. As primitive characters we consider the following: the membranous well-developed and persistent annulus, the uniform small cheilocystidia which do not entirely cover the edge of the lamellae, the decurrent gills, the geographic area which is enormous as compared with that of any of the other species which must have developed from a form like *K. mutabilis* by way of local races, and finally the wide variety of hosts, which shows a complete lack of specialization beyond the limitation to woody substrata.

2. **Kuehneromyces rostratus** sp. nov. FIGS. 6 & 9

Pileo cinnamomeo vel argillaceo-cinnamomeo-brunneo, hygrophano, clare alutaceo in siccis, ad marginem striatulo in udis, convexo vel plano obtuso, 20–60 mm. lato; epicute ex hyphis hyalinis filamentosis subparallelis, subgelatinescentibus, jacentibus efformata. Lamellis pallidis vel carneo-alutaceis in juvenilibus, cinnamomeo-argillaceis vel cinnamomeis in adultis, adnexo-sinuatis vel adnato-rotundatis, saepe dente decurrentibus, mediocriter latis vel latis, confertis; sporis 6–7.5 × 3.7–4.8 μ, eis *K. mutabili* simillimis, sed fortiter pluribus ellipsoideis quam ovoideis; basidiis tetrasporis; cheilocystidiis unius tantuin typi, elongatis, apice longissimo, tenui, parte ventricosa mediana vel

FIG. 9. *Kuehneromyces rostratus.* ×1.

inferiore, acumine haud noduloso vel rarissime in perpaucis noduloso, 41–62 × 2.4–9.3 µ: tramate lamellarum regulari, ex hyphis subintertextis pallidis (multis superpositis submelleis), elongatis consistente. Stipite aquose pallido vel subconcolori, quamquam pallidiore pileo sit, annulo apicali bene evoluto, angusto concolori, tenuimembranaceo, subpersistente praedito, squarruloso ad latus inferius annuli et infra annulum per nonnulla mm. ita ut in *K. mutabili* sed haud constanter nec tam distincte quam in illa specie, sericeo vel furfuraceo supra annulum, farcto dein cavo. Carne concolori superficiebus et plus minusve hygrophana, miti, odore nullo distincto. Ad sarmenta et ad ligna mortua arborum frondosarum fasciculariter et abundanter fructificans Maio mense in Milford, Mich., etiam in Takoma Park, Md., et Cleveland, O. Americae Borealis.—A speciebus aliis cheilocystidiorum apice elongato, plus minusve rostrato, tenui, nec non pileo obtuso, clare colorato in siccis et habitu eximie fasciculari differt.

Pileus 20–60 mm. broad, obtuse when young, soon convex to campanulate-convex, finally plane or depressed and sometimes the center perforated, rarely subumbonate, margin incurved at first, surface moist and hygrophanous, with a thin viscid or subviscid pellicle, glabrous at maturity, at first with fibrils along the margin, when young "clay color" to "Sayal brown," soon becoming "Pinkish buff" to "cinnamon," sometimes almost "avellaneous" when watersoaked, fading to warm buff or pallid and in dried specimens "warm buff" to "light buff" (darkest areas near "ochraceous buff"), or (in M. & P., **1930**, pl. 9, G–5), darkest spots "Inka gold," margin even at first, later translucent striatulate; flesh concolorous with surface and more or less hygrophanous, tapering outward evenly from the disc, taste mild, odor not distinctive; lamellae adnexed-sinuate to adnate and rounded, often with a decurrent tooth, moderately broad or broader, at least broader than context is thick and usually about 5 mm. near stipe which is the broadest part, close to crowded (50–60 reach the stipe), pallid to "pinkish buff" when young, near "clay color" or "cinnamon" when mature, thin, edges even; stipe 40–90 mm. long, 4–12 mm. thick, subequal or tapering upward from a ventricose midportion, the base usually tapering, characteristically long and fleshy, interior stuffed but soon hollow, surface watery-pallid or subconcolorous, but paler than pileus, with a distinct, narrow, apical annulus which is often evanescent, somewhat squarrulose-squamulose in the manner of *K. mutabilis* (but not so conspicuously so) on underside of annulus and for a short distance downward, usually glabrescent in age, silky to furfuraceous and whitish above the annulus, at the base appressed fibrillose from the white mycelial tomentum.

Spore deposit about "snuff brown"; spores 6–7.5 × 3.7–4.8 µ, at maturity rather well colored but remaining pale melleous when not fully mature (color as in *Flammula lenta*), ellipsoid to ovoid

(usually the former), terete or very indistinctly lentiform, with a double wall as in *K. mutabilis,* smooth, truncate; basidia about 21 × 6.5–7.5 μ four-spored; cheilocystidia 41–62 × 4.2–9.3 μ, in upper third 2–2.5 (3.5) μ, the apex very long in most, rostrate to rostrate-ampullaceous, the ventricose portion hyaline, tapered below ventricose portion (at or below the midportion) to a basal clamped septum, sometimes more fusoid than ventricose-rostrate-ampullaceous but even then very elongated and thin above, apex rarely nodulose and usually not forked or divided; gill edge typically heteromorphous because of very abundant cheilocystidia; pleurocystidia scattered or none; trama as in *K. mutabilis;* epicutis, hypoderm, and hyphae of context also as in *K. mutabilis.*

In woods on decaying frondose logs or debris, or on and around old sawdust piles or in areas where sawdust has been used as a fill. It has been found mostly on the wood or debris of *Quercus* or *Fagus.* It is very cespitose and fruits in May.

MATERIAL STUDIED: **Maryland,** Takoma Park, *C. H. Kauffman* (as *Pholiota* sp.) (MICH.). **Ohio,** Cleveland, *M. B. Walters* (MICH.). **Michigan,** Milford, *A. H. Smith 15002,* the type (MICH.).

The pleurocystidia are typically absent but frequently develop in areas where the hymenium has been injured. This same situation has been noted by the junior author in many species of *Psilocybe.*

3. **Kuehneromyces depauperatus** sp. nov. FIG. 1

Pileo sordide melleo, hygrophano, pallide carneo-alutaceo in exsiccatis, striatulo in udis, glabro, levi, convexo, subumbonato. Lamellis olivescente-fuscis in maturis, mediocriter latis, subconfertis, adnexis vel adnatis; sporis 6.2–6.8 × 3–4.4 μ, melleis, ellipsoideis vel ovoideis, truncatis; basidiis tetrasporis; cheilocystidiis 27–29 × 6–77 μ ampullaceis. Stipite atrofusco, imprimis ad basin, annulo supero appresso, pallido instructo, infra annulum subfibrilloso, subaequali, cavo, cc. 23 × 2 mm. Carne inodora. Ad truncum frondosum, muscosum, putridum in dumeto depressionis calcareae, solitario, Julio mense; Devil's Millhopper, Florida, U.S.A.— A *K. rostrato* colore pilei exsiccati, fructificatione solitaria aestivali et forma cheilocystidiorum differt.

Pileus about 17 mm. broad, subumbonate, smooth, convex, glabrous, translucent-striate when moist, non-striate when dry, deep sordid melleous when watersoaked, strongly hygrophanous and almost subviscid, fading to a pale pinkish buff when dry, becoming "pale yellow orange" to "light ochraceous buff" (R), or Pl. 9, F–5 (M. & P); context subconcolorous with the surfaces, watery and fleshy in pileus, somewhat tougher in the stipe; odor none; lamellae olive-fuscous when mature, medium broad (2–2.5

mm.), moderately close (about 23 reach the stipe), adnexed to adnate; stipe blackish fuscous, especially deep colored below, with a pallid fibrillose appressed apical annulus, subfibrillose below the annulus, subglabrous above it, white mycelioid at the base, subequal, narrowly hollow, about 23 × 2 mm.

Spores 6.2–6.8 × 3–4.4 μ, deep honey color, ellipsoid, some more ovoid, in frontal view often both sides more or less flattened (subrhomboid) and the diameter 0.2–0.4 μ larger than in profile, however never so distinctly lentiform as in *Deconica,* in lateral view less convex on the inner side, without suprahilar depression, with double, rather thick wall consisting of an uncolored endosporium and a colored exosporium of about equal diameter, with broad flatly truncate germ pore, smooth; basidia 14–20 × 5.8–6.3 μ, four-spored; cheilocystidia 27–29 × 6–7 μ, ventricose below, ampullaceous above (the neck 2–4 μ thick), sometimes slightly subcapitate sometimes cylindric to subconic above, hyaline, rather uniform in shape, size, and distribution; pleurocystidia none; trama regular, of dense, interwoven, melleous-hyaline hyphae; epicutis and hypoderm as in *K. mutabilis;* all hyphae with clamp connections.

On decaying, mossy trunk of a frondose tree in a lime-sink hammock vegetation (no conifers), solitary, fruiting in July.

MATERIAL STUDIED: **Florida,** Devil's Millhopper, Alachua Co., *R. Singer F 2992* (FH), type.

This species seems to be closest to *K. rostratus* from which it differs in the color of the stipe, in the more carneous shade of the dried pileus, the solitary manner of growth, the smaller stature, the shorter cheilocystidia, and the fruiting season. However, we do not know whether the latter difference is constant since it is possible that *K. depauperatus,* which is rather rare, has been overlooked by collectors during other seasons. On the other hand, the Florida seasons are so different from the northern fruiting periods that we cannot but attribute a certain importance to it in this case, at least until further data show that the Florida species is not restricted to the summer rainy season.

4. **Kuehneromyces vernalis** (Peck) comb. nov. FIGS. 7, 10

Agaricus vernalis Peck, Ann. Rep. N. Y. State Cab. **23**: 91. 1872.

Agaricus lignicola Peck, Ann. Rep. N. Y. State Cab. **23**: 91. 1872.

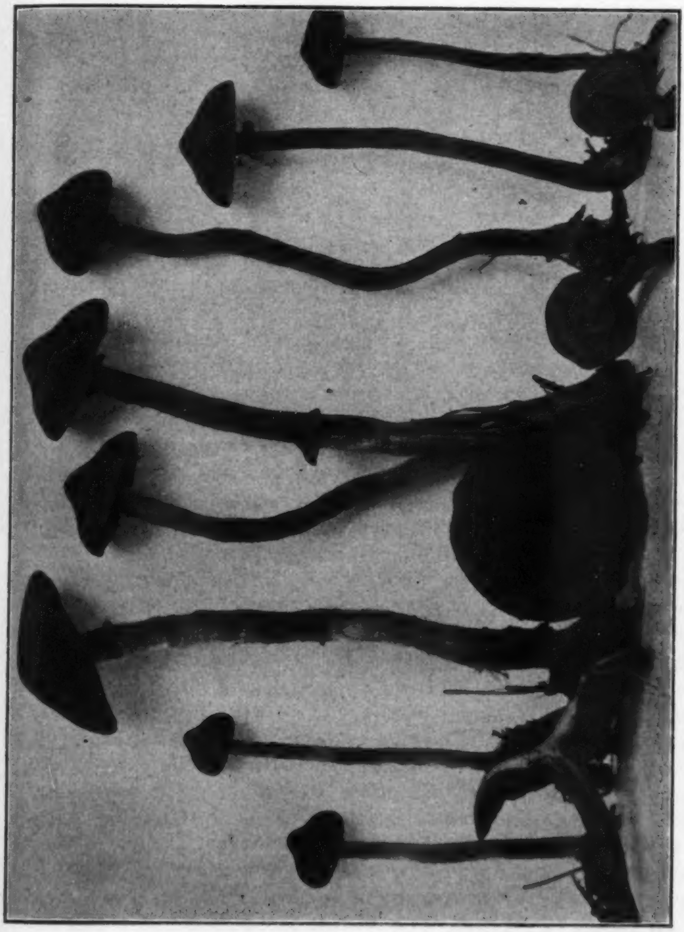

FIG. 10. *Kuehneromyces vernalis.* × 1.

Naucoria vernalis Saccardo, Syll. Fung. **5**: 838. 1887.
Naucoria lignicola Saccardo, Syll. Fung. **5**: 838. 1887.
Pholiota marginella Peck, Ann. Rep. N. Y. State Mus. **51**: 289. 1898.
Naucoria praecox Murrill, N. Am. Flora **10**: 174. 1917.

Pileus 8–35 mm. broad, convex to obtuse when young, the margin bent inward at first, soon expanding to plane or slightly depressed around the disc, margin sometimes remaining decurved, sometimes with a conic umbo or merely papillate and with a decurved margin, surface lubricous to subviscid when moist, glabrous except for fibrils or fibrillose patches on or near the margin from the broken veil, when moist with crowded translucent striations at least along the extreme margin, hygrophanous to subhygrophanous, darkest when young ("Dresden brown," "buckthorn brown" to "clay color" and becoming "yellow ocher," "chamois," "honey color" or paler before fading), often dusted "cinnamon brown" on the margin or over all from the spores, fading from the disc outward and finally changing to "maize yellow" or "warm buff" or between "warm buff" and "light buff," sometimes showing the close marginal striations when dry as well as when moist; flesh pale yellowish to subconcolorous with surface, somewhat hygrophanous, soft and typically fragile in the pileus, moderately thick in disc but very thin toward the margin, odor slightly raphanoid to almost lacking, taste mild to somewhat mawkish, not distinctly bitter in typical form; lamellae narrow (1.5–3.5 mm. in typical specimens but sometimes up to 5 mm. broad), adnate but readily seceding at least in age, sometimes subsinuate or narrowly adnexed, if not seceding, sometimes developing a decurrent tooth, but not as decurrent as in most specimens of *K. mutabilis*, very crowded to nearly subdistant (± 30 lamellae), arcuate to horizontal at maturity, "Sayal brown" to pale argillaceous but becoming dull rusty brown from the spores, edges entire but minutely white fimbriate (in specimens not past maturity); stipe 30–65 mm. long, 2–8 mm. in diameter, equal or tapered slightly either way, stuffed becoming hollow, sordid buff or tan to concolorous with pileus above, deeper brown below or soon becoming so ("clay color" young and fresh, soon "russet" to "mummy brown" from base upward), below the annulus (or superior fibillose zone) naked and silky-striate longitudinally, the thin fibrillose covering paler than the ground color, with a thin submembranous to fibrillose veil which leaves a thin superior annulus or fibrillose zone, surface above this zone fibrillose-pruinose, veil sordid-pallid to sordid-brownish.

Spore deposit varying from between "cinnamon brown" and "Verona brown" to between "snuff brown" and "Brussels brown" (pl. 15–E 12 of Maerz & Paul), the differences in color probably depending on age of spore print or the moisture content when fresh; spores 5.5–7.8 (10.2) × (3) 3.3–4.8 (5.5) μ, most frequently 7–7.5 × 4–4.5 μ in constantly four-spored forms, deep honey-color to melleous, fulvous-melleous in aggregations, ellipsoid to subamygdaloid in outline but a small percentage ovoid, rarely subrhomboid, either terete or very slightly lentiform (but not as compressed as in *Deconica*), in lateral view more convex on outer than on inner side, without suprahilar depression, with a double wall, consisting of a colorless endosporium and a colored exosporium, both about equally thick, smooth, with a very distinct, broadly truncate germ pore; basidia 17–25 × 8–7.3 μ, usually all four-spored but in some populations consistently mixed with two-spored basidia (and then the spores extremely variable in size and shape); cheilocystidia of two types, usually both present, but those of type II rare at times: type I abundant to the degree of making the gill edge heteromorphous, elongate in shape, hyaline or brownish only at base, the majority with a rather broad apical portion or apex subcapitate, midportion slightly ventricose (or enlargement either slightly above or below the middle), elongated neck often flexuous or flexuous throughout, rarely forked above, subampullaceous to subfusoid, thin walled or wall rarely 0.7 μ in diameter, 25–51 × 6–9.3 (12) μ, mostly around 45 × 7 μ, some in each mount broader than 3.5 μ in upper third: type II variable in abundance and distribution, sometimes scattered on the faces near the gill edge, typically broader than type I, vesiculose to vesiculose-clavate, balloon-shaped, up to 15 μ broad, sometimes with a short mucro, lower portion frequently brown, more rarely the whole either hyaline or brownish; subhymenium of densely and intricately interwoven hyphae characterized by a brown incrusting pigment; gill trama regular, with wart-like melleous pigment incrusted on hyphae of most carpophores; cuticle of pileus consisting of an epicutis and a hypoderm, the former consisting of a thin layer of hyaline, horizontally arranged strictly filamentous, narrow, subgelatinous to gelatinous hyphae, the hypoderm differentiated from the trama of the pileus merely by being pigmented (yellow to ocher brown) and consisting of slightly less voluminous elements, the hyphae often short and contorted (in sections creating the false impression of being intermixed with sphaerocysts), walls typically thin but sometimes appreciably thickened in age (apparently on material collected in dry weather); dermatocystidia none; all hyphae with clamp connections.

HABITAT: On sticks and logs, stumps and trunks, on small pieces of bark and on buried wood, often on decaying boards and beams, especially on bridges, the substratum either naked or covered by moss or earth. It is usually found on the wood of conifers (cedar, pine, larch, spruce, fir, and hemlock) but is known also from poplar and on *Fagus* and *Acer* as well as other kinds of frondose trees. In habit it is usually densely cespitose to fasciculate when at the peak of its fruiting period under very favorable conditions, but is often found in groups of only a few carpophores or even single at less favorable times. It is not as fasciculate as *K. mutabilis* or *K. rostratus*. It fruits from May until August or September, exceptionally later, and is common in the northern part of North America, rare otherwise. In North America it is known from New England west to the Pacific Coast and south to Tennessee. It is known from one station in northeastern Europe and from two in the Caucasus Mts. The distribution in general appears to be boreal and circumpolar.

MATERIAL STUDIED (including all aberrant forms, see the following): **Maine,** Canton Point, *J. C. Parlin, 17138, 17115* (both FH); **Vermont,** Middlebury, *E. A. Burt* (as *Naucoria lignicola*), Burt Herb. (FH); **New Hampshire,** Chocorua, *W. G. Farlow* (as *Pholiota marginata*) (FH); *R. Singer* (FH); **Massachusetts,** Harvard, *Harrie Dadmun* (FH); **New York,** types of *A. vernalis, A. lignicola,* and *P. marginella* (N. Y. S.); **Ontario,** Lake Timagami, Paradise Bay, T. F. R., *J. W. Groves* (det. *L. O. Overholts* as *Pholiota marginella*) (FH); **Michigan,** Emerson, *A. H. Smith 1304* (MICH.); Harbor Springs, *A. H. Smith 1278* (MICH.); Rock River, *A. H. Smith 33–44* (MICH.); Rees' Bog, Cheboygan Co., *A. H. Smith 1278* (MICH.); Higgins Lake, *A. McCrea* (MICH.); Wilderness State Park, *A. H. Smith 3282* (MICH.). **Tennessee,** Greenbrier, Sevier Co., Great Smoky Mts. National Park, *J. P. Porter* (det. *A. H. Smith*) (MICH.). **Wyoming,** Little Brooklyn Lake, *F. Arenberg 68* (det. *A. H. Smith*) (MICH.). **Idaho,** near Lewiston, *Wm. B. Gruber 22* (det. *A. H. Smith*) (MICH.); opposite Granite Creek, Bonner Co., *A. W. Slipp 1498* and *1513* (det. *A. H. Smith*) (MICH.); north of Gold Creek, Bonner Co., *A. W. Slipp 1591* (det. *A. H. Smith*) (MICH.). **Washington,** Jackson Guard Station, Olympic National Park, *A. H. Smith 13399* (MICH.); La Push, *A. H. Smith 12085* (MICH.); Hoh River, *A. H. Smith 13520* (MICH.); Clallam Bay, *A. H. Smith 13784* (MICH.); Port Ludlow, *A. H. Smith 13869* (MICH.); **Oregon,** Mt. Hood, *Wm. B. Gruber 3* (det. *A. H. Smith* (MICH.)). **U.S.S.R.,** Kola Peninsula, *L. A. Zinova* (det. *R. Singer,* as *Pholiota marginella* Pk.) (LE); Finno-Karelian Rep., Kivacz, *R. Singer* and *M. Freindling* (det. *R. Singer* as *Deconica acutiuscula* ined.) (LE); **Caucasus Mts.,** north slope, Czernoreczye, *L. N. Vassilieva* (det.

R. Singer as *Pholiota marginella*) (LE, KAZ); south slope, Babasch, Nakra Valley, *R. Singer* (as *Galera ravida*, see Beih. Bot. Centralbl. **48** (II): 530. 1931) (W or LE).

The indication of the above specimens is based on the assumption that *K. vernalis* is a very variable fungus, a fact that can be appreciated if three of Peck's own species are all considered to be synonyms of it. In some collections the annulus is distinct and the lamellae moderately broad. These are the "marginella type." Some have slender stipes and an almost fibrillose veil, the "*Naucoria* type." In those having variable spores the basidia are two- and four-spored. In some cheilocystidia of type II are abundant (f. *amara* and forms with mixed two- and four-spored basidia). In some the cheilocystidia appear capitate because of an adhering globule of viscous material—a feature very common in species of *Deconica* and one which should be observed on dried specimens revived in KOH—and in these the lamellae are crowded to close and the taste mild (f. *typica*) or bitter (f. *amara*). All these characters are intergrading, or so uncorrelated that we believe they should not be used at the species level here. Considering the strong variability of even a single collection (Chocorua, Singer), we can see no basis for recognizing *P. marginella* even as a variety of *K. vernalis*. The chief differences are a better developed veil, more obtuse pileus and broader lamellae. Judging from the type specimens of *Agaricus lignicola* and *Agaricus vernalis* both represent forma *typica*—no points of difference were found in a comparison of the types. It should be mentioned here that the European and Asiatic specimens cited have not been available for restudy in connection with this work. Notes of the senior author have been relied upon. A complete description of the Kivacz and Babasch specimens was made at the time the material was collected and reads almost word for word, including data on cheilocystidia, with that given here. The vesiculose cheilocystidia were noted as well as the others which were nodulose above. The senior author had planned to erect a new genus for these collections but still had doubts as to the exact color of the spore deposit. In his notes on one Washington collection (Port Ludlow), Smith also raised the question of the color of the spore deposit.

A number of variants appear to us to be worthy of designation

as formae. Forma *variabilis* is characterized by the mixed two- and four-spored basidia and great variation in spore size and shape. The spores measure 6.2–9.5 × 3.3–4 μ and on the average are very narrow. This is represented by Burt's collection from Vermont. His notes, however, do not indicate even the slightest difference between this and the type form in the macroscopic characters. The material was pressed and as a result appears slightly different, as if the specimens had been more slender in all parts. In his notes Burt described the lamellae as very crowded, joined to the stem by more than half their breadth, in having a denticulate edge (as is always true of *K. vernalis* if a good lens is used), and in not reaching the margin of the pileus (an unimportant character in this genus).

Forma **amara** f. nov. A forma typica sapore amaro et pileo lato, crassiusculo, stipite fortiore, lamellisque latiusculis differt. Little Brooklyn Lake, Wyoming, U. S. A. *F. Arenberg* 68, type.

As for the taste, we have to depend on Miss Arenberg's statement, but the generally heavier stature of these specimens and relatively broad gills may also be found to be correlated with taste and render the form easily recognized in the field. Studies of more collections are needed here. Cheilocystidia of type II are very prominent and well represented.

Forma **marginella** (Peck) comb. nov. (*Pholiota marginella* Peck, l.c.). This differs from the above form in absence of bitter taste, medium size and relatively well developed annulus. The type and some of the Maine specimens belong here. In this form pleurocystidia of type II are poorly represented. We doubt if this difference is constant between f. *marginella* and f. *amara,* but here more collections are needed. We have seen only a few. In f. *typica* the cheilocystidia are not constant in this character.

FIG. 10 illustrates non-cespitose fruiting bodies with long stipes. Smith (**1941**), pl. 7, illustrated more or less clustered carpophores.

The *Naucoria vernalis* of Atkinson (**1900**: 154, fig. 146) is certainly not Peck's species. As for *Pholiota marginella* sensu Overholts, it is difficult to state that this is precisely *K. vernalis* f. *marginella* and nothing else. It might include *K. rostratus,* Overholts did not describe the cheilocystidia. *Naucoria lignicola*

sensu Kauffman is not correct. The specimens filed under that name appear to belong in *Galerina.*

Pleuroflammula Singer gen. nov. Pileo minuto admodum excentrice vel lateraliter stipitato vel subsessili et stipite minusculo vel absente a genere *Flammula* (sensu stricto) et sporarum colore poroque exiguo a *Melanoto* differt. Species typica: *P. Dussii* (Pat.) Singer (*Crepidotus Dussii* Pat.).

Pleuroflammula Dussii (Pat.) Singer, comb. nov. FIGS. 4–5.
Crepidotus Dussii Patouillard, Bull. Soc. Myc. Fr. **18**: 173. 1902.

Pileus "colonial buff" to "cream color" or "ochraceous buff" to "amber brown," yellower near margin, browner toward disc, "antimony yellow" at margin, "Sudan brown" on disc in age, the covering forming a sterile narrow margin beyond the outer end of the lamellae, the surface of the pileus later glabrescent but in youth constantly fibrillose-subtomentose to tomentose, non-viscid, non-hygrophanous to subhygrophanous, smooth, convex, ellipsoid and attached at the broader side, or reniform, in larger specimens more often reniform than ellipsoid, the margin where it comes closest to the substratum always more or less attached to it and eventually becoming free, diameter 2–17 mm., most frequently between 4–10 mm.; lamellae almost "amber yellow" when seen from above in young specimens, the edge yellower than the sides which are more olive or more brownish than the edge, eventually becoming brown from the spores, comparatively broad (up to 2 mm. in the mature specimens), subclose to subdistant, adnexed, or when young also often emarginate-adnexed; spore print "Brussels brown"; stipe at apex similar in color to the margin of the pileus or the edges of the lamellae, below colored like the dorsal portion of the pileus, fibrillose-tomentose (or partially so) from the veil, later glabrescent at least over most of surface, sometimes indistinctly subannulate, always very small, curved, eccentric, later becoming sublateral and comparatively still more indistinct, up to 2×1.5 mm.; veil initially covering lamellae, later leaving a fibrillose-subtomentose sterile margin on pileus and a fibrillose-tomentose or fibrillose-flocculose covering on the stipe, sometimes forming a narrow, small, indistinct annulus at the line where the fibrils break as the cap expands, concolorous or paler than the margin of the pileus; context yellowish, bitterish.

Spores $6–9.3 \times 4.5–7.3\,\mu$, the larger and more mature the broader they become, tawny-ferruginous-melleous, without depression, smooth, with a double wall which is not entirely uninterrupted at the apex but the apex not truncate and there is no easily

discernible germ pore (or it is indistinct); basidia about 26 × 6.3–7 μ, four-spored; cheilocystidia very distinct, readily visible in all stages of development, hyaline ventricose-ampullaceous, 30–55 × 8–10.2 μ, thin-walled, the neck broad and broadly rounded above, not incrusted, very numerous and making the edge of the lamellae completely heteromorphous, occasionally some cheilocystidia found slightly back from the edge but no true pleurocystidia seen; trama of lamellae regular; cuticle of repent hyphae with pigment incrustations; often a yellow coloring matter exuded when preparations of the hymenophore are crushed in ammonia; all hyphae with clamp connections.

On limbs and twigs of dicotyledonous trees and shrubs, often seen on *Liquidambar styraciflua* and *Viburnum obovatum* in Florida, growing on fallen wood as well as on dead portions of the standing tree or shrub, often high up on the trunk or branches, solitary or gregarious, in Florida fruiting in June, July and August.

This species was described originally from the Bois des Bains Jaunes, Guadeloupe, W. I., where Duss collected it "in small quantity on a pile of indeterminable wood; color yellow, no. 411." Patouillard published it as *Crepidotus Dussii* but the spores are not those of a *Crepidotus* nor are the colors of the carpophores and the veil characteristic for any group of species or any single species ever recognized in *Crepidotus*. The spores in that genus are less fulvous and the wall is continuous at the apex; the pigments of *C. Dussii* are those of a *Flammula* rather than of any other ochrosporous agaric.

Another species of the genus *Pleuroflammula* has been described under the name of *Crepidotus flammeus* Murr. I have seen the type specimen from Virginia and a specimen from the Brogdon Hammock, Dade County, Florida. Both were determined by W. A. Murrill, and were kindly put at our disposal by Dr. Fred J. Seaver. They are very close to *Pleuroflammula Dussii*, especially macroscopically—though perhaps still more brightly colored—but differ microscopically in slightly larger spores with less developed germ pore, and, more important, in longer and narrower cheilocystidia which are capitate above, and have a yellow to brownish-melleous incrustation. The binomial **Pleuroflammula flammea** (Murr.) Sing. comb. nov. is proposed for this species.

Although not generally in favor of recognizing as separate gen-

era groups distinguished from existing genera only by their habit (here shape of the stipe) the author thinks that in the present case we have a repetition of an analogous case in the Cortinariaceae where a tropical genus with strongly eccentric to lateral, often strongly reduced stipe, *Pyrrhoglossum*, is opposed to a genus with more or less central stipe and wide distribution all over the climatic zones of all continents, *Gymnopilus*. In both cases, the pleurotoid genus is much smaller in size of the fruiting bodies as well as the number of species. The hiatus between the centrally stipitate group and the pleurotoid group in both cases, *i.e.*, between *Pyrrhoglossum* and *Gymnopilus,* and between *Pleuroflammula* and *Flammula,* is sharp and distinct enough to separate two autonomous though closely related genera. However, whereas *Pleuroflammula* and *Pyrrhoglossum* are not actually related to each other, *Pleuroflammula* is systematically analogous to *Melanotus* and certainly belongs to the same family as the latter. It represents the hitherto missing link in the two parallel series of rusty and dark-spored agarics, here combined as Strophariaceae.

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FUNGI NOVI DENOMINATI—II

John A. Stevenson

The following fungi received from various sources appear to be new and are herewith named and described in continuation of a previous series (Mycologia **35**: 629–637. 1943). Types of each have been deposited in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, at Beltsville, Maryland.

Physoderma paspali sp. nov.

Maculis brunneis, limitatis, amphigenis, ovalibus vel angularibus, 4–8 mm. long.; *sporangiis* globosis, subglobosis vel breve cylindraceis, utrinque rotundatis, 18–33 × 15–24 µ; *episporio* 1–1.5 µ crasso.

Causing amphigenous spots on leaf blades, few to many and more numerous near juncture of blade with sheath, oval or angular to somewhat irregular and elongated, sometimes coalescing, 4–8 mm. long, chocolate brown at first (near russet of Ridgway), becoming ashen with a diffuse gray-purplish surrounding area, shredding with age, disclosing under a hand lens yellow brown masses of sporangia imbedded in the leaf tissues; *sporangia* spherical, subspherical or short cylindrical with ends rounded, smooth, golden brown, 18–33 × 15–24 µ; *wall* 1–1.5 µ thick.

In living leaf-blades of *Paspalum plicatulum* Michx. (Gramineae), Rio Piedras, Puerto Rico, *John R. Johnston 4445,* June 1912; *John A. Stevenson 164,* Oct. 1913; *6212,* Feb. 1917; *6549,* June 1917; *6910,* March 14, 1918; Myc. Coll. *71415,* March 15, 1918 (Type).

This fungus causes a definite, well marked spotting of the host leaves. In advanced cases the spots are frequently overgrown by *Colletotrichum graminicolum* (Ces.) G. W. Wils. Bitancourt has reported *Physoderma* sp. on *Paspalum millegrana* Schrad. in Brazil (Inter. Bull. Plant Prot. **12**: 52 m. 1938), but without descriptive notes. Other species of *Physoderma* on grasses such as *P. zeae-maydis* Shaw and *P. gerhardti* Schroet. differ in smaller sporangia and other distinctive characters.

Ustilago speculariae sp. nov.

Soris in capsulis; sporis nigris, opacis, levibus, globosis vel subglobosis, 21–27 μ diam.

Sori in capsules of host, causing slight distortion, upon rupture of enclosing tissues disclosing black dusty spore-mass; spores black, opaque, smooth, globose to subglobose, sometimes more irregular or elongate, 21–27 μ diameter.

In capsules of *Specularia perfoliata* (L.) A. DC. (Campanulaceae), McAlester, Pittsburg Co., Oklahoma, *F. W. Pennell* (*10592*), May 27, 1920. (Type, Myc. Coll. *71423*.)

This smut appears to be rare, which is perhaps in part accounted for by the fact that the clasping leaves of the host effectively conceal the aborted capsules which differ very little in size or color from normal ones. No other records have been found of the occurrence of smut species on the genus *Specularia* nor in fact on the family Campanulaceae.

Entyloma trigonellae sp. nov.

Soris amphigenis, maculis rotundatis vel ovalibus, minutis, 1–2 mm. diam., luteolis; *sporis* sparsis, globosis vel subglobosis, hyalinis vel flavescentibus, levibus, 12–16 μ diam.; *episporio* 1.5–2 μ crasso; *conidiis* non visis.

Sori in leaves, not abundant, amphigenous, forming minute spots, 1–2 mm. diameter, circular to oval, light yellow, sometimes with a greenish halo upon drying; *spores* sparse, spherical to subspherical, rarely ovoid, hyaline to yellowish, often with evidences of gelatinous envelope which in some cases forms papillae on the spore wall, otherwise smooth, 12–16 μ diameter, rarely 12–18 $\times$ 14–15 μ; *spore wall* 1.5–2 μ thick; no conidial development noted.

On living leaves of *Trigonella foenum-graecum* L. (Leguminosae), Davis, California, comm. *Max W. Gardner*, May 1935. (Type, Myc. Coll. *71405*.)

Asterina (Englerulaster) phoradendricola Stevenson and Pollack sp. nov.

Coloniis amphigenis, nigris, circularibus, 1–5 mm. latis, vel confluentibus; *hyphis* reticulatis, brunneis, 4.5–6 μ diam.; *hyphopodiis* paucis, irregulariter dispersis, non-septatis, sessilibus, hemisphaericis vel breve cylindricis, late rotundatis, 7–10 μ longis, 5–7 μ latis; *thyriotheciis* numerosissimis, circularibus,

vel confluentibus, pulvinatis vel hemisphaericis, 60–100 μ diam.; *strato tegenti* atro-brunneo, ex hyphis radiantibus irregulariter dehiscentibus composito; *ascis* sessilibus, late ovatis vel subsphaericis, aparaphysatis, octosporis, 40–48 μ diam.; *sporis* oblongis, valde constrictis utrinque late rotundatis, levibus, atro-brunneis, 26–32 × 12–16 μ; *cellulis* subglobosis, superiori 14–16 μ inferiori 12–14 μ latis.

Colonies amphigenous, black, circular to somewhat irregular, up to 5 mm. in diameter, frequently confluent and covering large areas of leaf surface; *mycelium* scanty, "cinnamon brown" to "sayal brown," reticulate, meshes angular; *hyphae* straight, branching irregularly, 4.5–6 μ in diameter, with cells 15–30 μ in length; *hyphopodia* few, scattered, alternate or unilateral, sessile, hemispherical to short subcylindrical, broadly rounded above, non-septate, 7–10 μ long, 5–7 μ broad; *thyriothecia* very numerous, gregarious, often confluent in small groups, uniformly distributed over entire colony, circular to subcircular in outline, pulvinate to hemispherical, 60–100 μ diameter; *covering membrane* dark brown, composed of radiating hyphae which dehisce irregularly early in their development and are replaced by dark colored slime which encloses strands of spherical to elliptical brown cells; *basal layer* composed of radiating pale brown hyphae; *asci* one to many in a thyriothecium, sessile, thick walled, broadly ovate to spherical, 8-spored, aparaphysate, 40–48 μ diameter; *spores* oblong, broadly rounded at both ends, strongly constricted at the septa, almost equally uniseptate, smooth, finally dark brown, but long remaining hyaline, 26–32 × 12–16 μ; *cells* subglobose, upper 14–16 μ broad, lower 12–14 μ.

On living leaves of *Phoradendron flavescens* (Pursh) Nutt. (Loranthaceae), parasitic on *Carya pecan* (Marsh.) Engler & Graebn., Newnan's Lake, near Gainesville, Alachua Co., Florida, *Arthur S. Rhoads,* Nov. 11, 1943. (Type, Myc. Coll. no. *71427*.) Fifteen additional collections were made by the same collector from September 1943 to February 1944 from the same host found parasitizing various species of *Nyssa, Planera, Prunus, Quercus,* and *Xanthoxylum* in Alachua, Highlands, Lake, Marion, Putnam, and Volusia Counties, Florida. (Myc. Coll. nos. *71428–71442*.)

This species appears to be abundant and widespread, at least in the state of Florida, on the common American mistletoe. It is characterized by the numerous dull black amphigenous colonies which are often confluent to such an extent as to cover much of the leaf surface. The fungus is marked by the breaking down of the covering membrane of the thyriothecium into a dark colored mu-

cilaginous slime in which chains of spherical to elliptical brown cells occur. This character places the species in V. Hoehnel's *Englerulaster,* a genus which later workers have considered more appropriately placed as a section of *Asterina,* a decision in which we concur. The present species differs from others named on the *Loranthaceae,* including *Asterina loranthicola* Syd. and *A. loranthacearum* Rehm, by the *Englerulaster* character and by the spore sizes. *A. phoradendri* P. Henn. has been referred to *Asterinella* by Theissen, because of the absence of hyphopodia.

Meliola condaliae sp. nov.

Coloniis amphigenis, circularibus, 2–3 mm. diam.; *hyphis* 6–8 μ diam., ramis plerumque oppositis; *hyphopodiis capitatis* alternantibus, oppositis vel unilateralibus, 10–15 μ longis, 6–7 μ diam.; *hyphopodiis mucronatis* sparsis, oppositis vel unilateralibus, 15 μ longis; *peritheciis* globosis, 150–200 μ diam; *ascis* evanidis, bisporis; *sporidiis* quadriseptatis, levibus, obtusis, 37–45 × 12–18 μ; setis rectis, opacis, usque 400 μ, basis 6–7 μ crassis, acutis vel duobus-pluribus dentatis.

Colonies amphigenous, but more numerous above, circular, 2–3 mm. diameter, at times coalescing to cover entire leaf surface, more rarely on smaller twigs; *mycelium* branching commonly opposite, but may be unilateral or irregular, dark brown, straight, 6–8 μ diameter (cells 15–24 μ long); *capitate hyphopodia* predominantly alternate, but some opposite and unilateral, short cylindrical to narrowly ovate, 10–15 μ long, 6–7 μ diameter, *head cell* narrowly ovate, 6–7 μ diameter; basal cell very short (2–3 × 4–5 μ); *mucronate hyphopodia* few, ampulliform, opposite or unilateral, 15 μ long, 5 μ diameter (at base); *perithecia* grouped in center of colonies, spherical, finally depressed flattened, verrucose, 150–200 μ diameter; *asci* evanescent, two-spored 45–50 × 24–30 μ; *spores* quadriseptate, deep brown, smooth, somewhat constricted at septa, obtusely rounded at both ends, 37–45 × 12–18 μ; *mycelial setae* uniformly scattered over colonies, straight or somewhat flexuous, opaque, 250–400 μ long, 6–7 μ diameter at base, acute at tips, notched or with two to several teeth up to 12 μ long. Formula under the Beeli system 31⅓ 3.4222.

On living leaves and twigs of *Condalia obovata* Hook. (Rhamnaceae), Brownsville, Cameron Co., Texas, *Robert Runyon 3663* (Type), Feb. 1944; same locality, *C. J. Hansel 57475,* March 1944; Harlingen, Cameron Co., Texas *R. Runyon 4115,* Dec. 1945; Matamoros, Mexico, *D. J. Smith 59943,* Dec. 1945.

No previous reports have been found of any species of *Meliola* on the genus *Condalia*. On the other genera of the Rhamnaceae several species are described, but all differ in essential characters. *Meliola rhamnicola* Stevens and Tehon described from British Guiana on *Gouania* differs in its diffuse colonies, capitate hyphopodia which are alternate only, and in the smaller spores. *Meliola scutiae* Speg. described from Argentina on *Scutia* is even more divergent with opposite hyphopodia, smaller spores, undivided setae tips, larger perithecia and shorter setae.

Phyllosticta malvavisci sp. nov.

Maculis circularibus, amphigenis, 2–4 mm. diam., cinereis, atro-brunneo-marginatis; *pycnidiis* epiphyllis, membranaceis, 100–140 μ diam.; *conidiis* hyalinis, globosis vel ovalibus, granulosis, 6–9 × 5–7 μ.

Spots mostly circular, occasionally oval to somewhat irregular, showing on both surfaces, but more distinct above, few to many per leaf, 2–4 mm. in diameter, with narrow dark brown border and ashen-gray center above, greenish to cinereous beneath; *pycnidia* numerous, chiefly epiphyllous, scattered uniformly over the spots, membranous, 100–140 mm. diameter; *conidia* hyaline, globose to oval and long ovate, granular, 6–9 × 5–7 μ.

On living leaves of *Malvaviscus drummondii* Torr. & Gray (Malvaceae), Brownsville, Texas, *C. J. Hansel,* Feb. 1944. (Type, Myc. Coll. *71411.*)

This fungus and the leaf spot which it causes appear quite distinct from other species of *Phyllosticta* previously named on species of Malvaceae. *Ph. altheina, Ph. hibiscina* and *Ph. hibisci* in particular differ markedly in character of the spots produced and in the shape and dimensions of the conidia.

Phleospora plucheae sp. nov.

Pycnidiis amphigenis, foliicolis, innatis, 120–150 μ diam.; *conidiis* hyalinis, aseptatis vel uniseptatis rectis vel curvulis, non constrictis, cylindraceis vel subclavatis, 15–40 × 5.5–7.5 μ.

Pycnidia few to many, amphigenous in slightly discolored irregular areas on leaves, flask shaped, 120–150 μ diameter, 120–200 μ in depth, deeply embedded in host tissue, opening by a wide pore (15–25 μ); *conidia* cirrhose, hyaline, very light pinkish in

mass, continuous or median uniseptate, variable in shape, cylindrical to long subclavate (approaching subfusoid), with obtuse ends, straight to curved, occasionally slightly sigmoid, not constricted at the septa, 15–40 × 5.5–7.5 μ, most commonly 28–32 μ in length.

On living leaves of *Pluchea sericea* (Nutt.) Coville (Compositae), Presidio, Texas, *J. H. Russell,* March 17, 1944. (Type, Myc. Coll. *71404.*)

This species is rather easily overlooked because of the lack of distinctly discolored necrotic areas and by the fact that such discoloration as does occur is masked by the pubescence of the host. The fungus appears to be none the less effective in bringing about defoliation. It differs markedly in its conidia from *P. baccharidicola* Speg., the only other member of the genus reported on Compositae. *Septoria plucheae* Guba is distinct for the same reason.

Septoria allardii Stevenson and Pollack, sp. nov.

Maculis orbicularibus, ovalibus vel angularibus, amphigenis, obscure fulvis dein cinereis et fulvomarginatis, subtus obscure griseo-viridis, 2–5 × 3–10 mm.; pycnidiis dense gregariis, amphigenis, membranaceis, immersis, globosis vel globoso-depressis, ostiolatis, 125–200 μ diam.; conidiis acicularibus, hyalinis, utrinque acutiusculis, non septatis, rectis vel curvatis, 30–45 × 0.5 μ.

Producing circular or oval to angular leaf spots, at times coalescent to form irregular blighted areas, particularly at the tips, amphigenous, dull brown becoming ashen with definite brown borders, dull gray-green beneath, 2–5 × 3–10 mm.; pycnidia amphigenous, numerous, uniformly developed over lighter colored portion of spots, membranous, globose to globose depressed, immersed in leaf tissue, ostiolate, 125–200 μ diameter; conidia acicular, hyaline, straight or somewhat curved, non-septate, acute at both ends, 30–45 × 0.5 μ.

On living leaves of *Melanthium parviflorum* (Michx.) S. Wats. (*Veratrum parviflorum* Michx.) (Liliaceae), Elliott Knob, Augusta Co., Virginia, *H. A. Allard,* June 3, 1934. (Type, Myc. Coll. *71443.*) The species is named in honor of H. A. Allard the collector and long connected with the botanical work of the Bureau of Plant Industry.

Septoria sublineolata Thuem. described from Russia on leaves of *Veratrum album* differs in many respects from our species, including epiphyllous, sparse pycnidia in elongated spots and par-

ticularly the size and shape of the conidia which are given as $60 \times 4\ \mu$, rounded-acute at both ends. *Cylindrosporium veratrinum* Sacc. and Wint. known on several species of *Veratrum* both in this country and Europe differs in the character of the fruiting bodies as well as in the linear leaf spots and the much larger, septate conidia.

Chaetoseptoria wellmanii sp. nov.

Pycnidiis amphigenis, paucis in quaque macula (3–10), membranaceis, 120–170 μ diam.; ostiolis definitis, circularibus, 15–25 μ diam.; setis rectis, erectis, 3–6 septatis, 5–6 μ diam., 90–225 μ longis; conidiis hyalinis, acicularibus, rectis vel curvatis, indistincte septatis, $75–160 \times 2.5–4\ \mu$.

Pycnidia few per spot (3–10), scattered, immersed, then partially erumpent, amphigenous, membranous, 120–170 μ diameter; *ostiole* definite, circular, 15–25 μ diameter; *ostiolar setae* straight, erect, brown, sparingly septate (3–6), 5–6 μ diameter, larger at the base (up to 9 μ), 90–225 μ long; *conidia* hyaline, acicular, straight or variously curved, obtuse to truncate at one end, the other long acute, sparingly and indistinctly septate, $75–160 \times 2.5–4\ \mu$.

On living leaves of *Phaseolus vulgaris* L. (Leguminosae), near La Ceiba, El Salvador, July 1, 1943, *F. L. Wellman 126a* (Type), *128;* Sacocoyo, El Salvador, July 3, 1943, *136, 137;* on living leaves of *Vigna sinensis* Endl. (Leguminosae), near Sacocoyo, July 3, 1943, *1940;* Zapitotan, Aug. 5, 1943, *424*. The fungus is associated with circular to somewhat irregular leaf spots, commonly not over 2–6 per leaflet, and up to 1 cm. in diameter, which are occasionally coalescent, dull brown in color at first, but becoming ashen at the center with an indefinite outer dull brown area without a definite margin and no appearance of zonation.

This fungus, which clearly belongs in Tehon's previously monotypic genus *Chaetoseptoria* (Mycologia **29**: 444–5. 1937), differs from his type species in a number of particulars, including amphigenous pycnidia and longer setae, but more especially in the conidia which are much longer and wider than in *C. vignae,* and are very sparingly and indistinctly septate if at all, with one end long acute. I am indebted to Dr. Tehon for an opportunity to study the type of his *C. vignae*, known at present only from Illinois.

Ovularia lupinicola Pollack [1] sp. nov.

Maculis amphigenis, circularibus usque irregularibus, 1–7 mm. diam., brunneis, arescendo bubalinis, margine rubro-brunneo, zona discolore circumdatis; *hyphis* hypophyllis, singulis v. fasciculatis, rectis v. flexuosis, hyalinis, continuis, interdum denticulatis; *conidiis* obovatis usque globosis, continuis, hyalinis, 16–32 × 12–20 μ.

Producing circular to irregular spots which are visible on both leaf surfaces, but more clearly above, 1–7 mm. in diameter, at first brown, finally buff at center (0.5–3 mm.) with reddish brown margin surrounded by a second buff to dark brown zone (up to 4 mm.); *fungus fruiting* usually hypophyllous, white; *conidiophores* single or in fascicles, hyaline, continuous, straight to flexuous, sometimes denticulate, bearing one or more scars where conidia have been attached, 20–90 × 4–8 μ; *conidia* obovoid to spherical, non-septate, hyaline, 16–32 × 12–20 μ.

On living leaves of *Lupinus* sp. (cf. *L. polyphyllus* Lindl.) (Leguminosae), East Stanwood, Washington, *R. F. Wilbur,* May 19, 1944. (Type, Myc. Coll. No. *71444*); *L. parviflorus* Nutt. ex Hook & Arn., Grand Mesa, Colorado, *R. W. Davidson 612,* July 12, 1930.

This is apparently the first record of an *Ovularia* parasitizing *Lupinus*. *Ovularia ? globifera* Ell. & Ev. reported on several species of *Lupinus* was transferred to *Hadrotrichum* as *H. globiferum* (Ell. & Ev.) J. J. Davis. *Ovularia sphaeroidea* Sacc. originally described on *Lotus corniculatus* in Europe is reported on *Lupinus* sp. in A. B. Seymour's Host Index of the Fungi of North America. Seymour's citation is based apparently on a record by S. M. Tracy and F. S. Earle (in E. L. Greene, Plantae Bakerianae, vol. 1, p. 35–36. 1901) of a specimen collected by them in Colorado in 1898 on "living leaves of Lupinus, Chicken Creek, 9000 feet, July 6, no. *368*" and listed as *O. sphaeroidea.* A study of a portion of the original collection as issued by Baker, Earle and Tracy in their "Plants of Southern Colorado" reveals that the fungus is again *Hadrotrichum globiferum* and not an *Ovularia.*

Septonema agaves sp. nov.

Caespitulis fuscis, late effusis in plagulis ovalibus v. irregularibus, hypophyllis; conidiophoris levibus, badiis, septatis, 30 × 3–4 μ; conidiis 1–9-

[1] Flora G. Pollack, Assistant Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering.

septatis, brevibus, catenulatis, in septis non constrictis, rectis, cylindricis, utrinque rotundatis, verruculosis, atrobadiis, 15–45 × 5–6 μ.

Fungus patches hypophyllous, dark brown, broadly effused over oval to somewhat irregular leaf spots; *conidiophores* smooth, light brown, branching, septate, up to 30 μ long, 3–4 μ diameter; *conidia* 1–9 septate, short catenulate, verrucose, deep brown, not constricted at septa, straight to slightly curved, cylindrical with both ends rounded, deep brown, 15–45 μ long, 5–6 μ diameter.

On living leaves of *Agave americana* L. (Amaryllidaceae), Lake Ilopango Road, El Salvador, *F. L. Wellman 300* (Type), July 25, 1943.

This species is constantly associated with very definite oval to somewhat irregular brown leaf spots which in dried material at least are somewhat raised and with a very definite border. The conidia are in short chains which break up tardily and since constrictions are very little in evidence, it is often difficult to decide just how many conidia go to make up the longer chains, which may reach a length of 150 μ.

Septonema agaves appears to be distinct from *S. olivaceo-nigrum* Berk. & Br. described from Ceylon, "apparently on leaves of *Agave*" by the very definite leaf spots and by the longer nonconstricted conidia.

Cercospora lonchocarpi sp. nov.

Maculis amphigenis, subcircularibus dein irregularibus et confluentibus, fuligineis, dein cinereis, fusco-marginatis; *mycelio* innato, castaneo, septato, 3–4 μ diam.; *conidiophoris* amphigenis, dense caespitosis, erumpentibus, brunneis, 20–60 μ longis, regularibus vel subflexuosis, stromatibus compactis enatis, continuis vel 1–2 septatis; 20–60 × 3–5 μ; *conidiis* obclavatis-acicularibus, subhyalinis, fumosis vel brunneis dilutis, indistincte septatis, rectis vel curvatis, 20–75 × 3–4 μ.

Spots amphigenous, at first approaching circular, but soon becoming very irregular and often confluent to form large dead areas involving much of leaf surface, dull brown at first, finally light tan to ashen, with a very definite narrow dark brown circumscribing line and a yellow-brown halo 3–5 mm. wide surrounding nonconfluent spots; *mycelium* internal, regular, septate, brown, 3–4 μ diameter, forming compact subepidermal stromatic areas, 30–60 μ in diameter; *conidiophores* amphigenous, densely to very densely tufted, rupturing the epidermis, regular to subflexuous, 20–60 μ

long, 3–5 μ diameter, brown, continuous or uni- to biseptate; arising from a compact tuberculate stroma, conidia obclavate-acicular, subhyaline to smoky or very light brown, indistinctly few to many septate, straight or curved, sometimes sigmoid, 20–75 × 3–4 μ.

On living leaves of *Lonchocarpus nicou* (Aubl.) DC. (Leguminosae), Belem, Pará, Brazil, *W. A. Archer H–464* (Type), Feb. 14, 1945; *L. nicou*, Quista Cocha, 17 miles west of Iquitos, Peru, *Bowen S. Crandall 3217a*, June 22, 1944; *L. urucu* Killip and A. C. Sm., Belem, Pará, Brazil, *W. A. Archer H–428*, July 6, 1942; *L. chrysophyllus* Kleinh. Wauna, Koriabo River, Brit. Guiana, *W. A. Archer H–249*, July 20, 1934.

This leaf-spot inducing fungus appears to be common and widespread wherever the host genus is grown in northern South America. Dr. Archer reports it as causing considerable defoliation at times and it may well become of economic concern in large plantings.

PLANT INDUSTRY STATION,
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SOME TAXONOMIC NOTES ON THE HIGHER FUNGI

LINDSAY S. OLIVE

(WITH 4 FIGURES)

This paper brings together a miscellaneous assortment of notes on various fungi collected by the author in different localities in this country. A few have been rarely collected and are redescribed here. A new *Tremella,* particularly interesting from the standpoint of its parasitism of another gelatinous Heterobasidiomycete, is described.

ASCOMYCETES

NADSONIA FULVESCENS (Nads. & Kon.) Syd. (FIG. 1: nos. 1–13)

This yeast was found by the author on May 30, 1945, at Beltsville, Maryland, in the sap of *Betula nigra* L., which was exuding from a wound high up on the tree and falling onto leaves lying on the ground. This appears to be the second report of the genus *Nadsonia* outside Europe. The yeast grew in the form of conspicuous white or cream-colored patches, which were foamy in appearance. Other fungi were mixed with the yeast, but the latter was the predominant species.

The writer's identification of the organism was confirmed by Dr. J. N. Couch, who (**1944**) redescribed the fungus and gave its history. His is apparently the first report of *Nadsonia* in this country. Couch found the yeast at Chapel Hill, North Carolina, growing in great abundance in the slime flux of recently cut birch trees.

The Maryland collection was obtained in pure culture on corn meal agar, on which it grew well in the form of smooth, creamy white colonies, and sporulated abundantly within a few days. Microscopic observation revealed the presence of budding vegetative cells (FIG. 1: nos. 1 & 2) mixed with numerous cells in the process of ascospore formation (FIG. 1: nos. 3–13). As previously described, ascospore formation is commonly preceded by the production of a

bud at each end of a cell, the middle cell becoming the female gamete, one of the buds becoming the male gamete, and the other bud the ascus (FIG. 1: nos. 3 & 4). The male gamete then empties into the female cell and both migrate into the ascus where a single ascospore is formed (FIG. 1: nos. 5, 6, 12). According to Nadson and Konokotine (**1926**), the process is accompanied by a nuclear fusion and meiotic division, only one of the four resulting nuclei persisting to become the nucleus of the single ascospore. These authors reported variations of this process in which two to four nuclei might persist, with the formation of an equal number of ascospores. I have also found variations in which two to four asci appear, each with a well developed ascospore (FIG. 1: nos. 7, 8, 10, 11). Sometimes a single ascus is found to contain two or three spores (FIG. 1: nos. 9, 13). The ascospores measure 4.5–5.4 μ in diameter. These dimensions fall within the measurements given in previous descriptions of the fungus.

MELANOSPORA INTERNA Tehon & Stout (FIG. 1: nos. 14–18)

During the writer's recent employment with the Emergency Plant Disease Survey of the United States Department of Agriculture, a shipment of diseased peanut pods from North Carolina was received from Dr. Alton E. Prince, also in the employ of the Survey. The material was collected in October, 1944. Peanuts in two of the collections were found to contain perithecia, both outside and inside the pods, of a fungus belonging to the genus *Melanospora*. No other fungal growth was apparent to the unaided eye at that time, but when the pods were placed in a moist chamber a species of *Fusarium* began to sporulate abundantly over the diseased areas. Whenever a transfer of the *Melanospora* to agar was attempted, the *Fusarium* invariably accompanied it, or else the transfer failed to develop altogether. Single spore cultures were attempted without success. Some ascospores, which had already begun to produce germ tubes when taken from a mixed growth, discontinued development when placed alone on nutrient agar. But whenever the *Fusarium* accompanied the transfer to the same agar medium, the *Fusarium* and *Melanospora* both grew, the latter producing numerous golden yellow perithecia plainly visible

throughout the white flocculent mass of *Fusarium* hyphae and usually well above the surface of the agar.

Thus it appears that the *Melanospora* is a parasite on the *Fusarium*. In culture the latter seemed to suffer very little damage from this relationship. Nothing is known about the possible influence of the *Melanospora* in checking growth of the *Fusarium* in nature, but it does not seem likely that it would have much effect upon it. It is obvious that the *Fusarium* is the important agent in causing pod-rot in the diseased peanuts.

A careful study of the *Melanospora* revealed that it is identical with *M. interna* Tehon & Stout (**1929**) which was described by these authors as occurring in rotting roots of tomato. Although the fungus was then believed to be the cause of this root-rot, experiments in culturing it would probably have shown that it was growing on a *Fusarium*, which was in turn the chief cause of the disease.

The following characteristics based on observations of the fungus on peanut pods as well as in mixed cultures with *Fusarium*, are here included for the benefit of those who might encounter it when studying diseases caused by *Fusarium*. Perithecia 125–300 μ in diameter; rostrum present, not setose, or with short setae at the apex, 35–50 × 34–110 μ; asci clavate, 20–24 × 53–72 μ; ascospores 8.5–10.5 × 20–23 μ, spindle-shaped, conspicuously guttulate, chocolate brown, outer wall often revealing a reticulation. In the first cultures of the fungus numerous hyphae with phialides which produced small globose phialospores were observed. These did not reappear in later cultures, but are believed to belong to the *Melanospora* life cycle. They have been reported by other investigators for various species of *Melanospora*. The characteristics given here agree very closely with those given by Tehon and Stout (**1929**) in

FIG. 1. *Nadsonia fulvescens* (nos. 1–13). 1, 2, budding vegetative cells; 3–6, stages in ascospore formation; 7–11, variations in ascospore formation; 12, one-spored ascus; 13, two-spored ascus. *Melanospora interna* (nos. 14–18). 14, hypha bearing phialides and phialospores; 15, perithecium; 16, group of mature and immature asci; 17, mature eight-spored ascus; 18, *a–e*, mature ascospores showing oil droplets; *f*, surface view of ascospore showing reticulated outer wall. (All drawings × 945, except 15, × 300, and 16 and 17, × 450.)

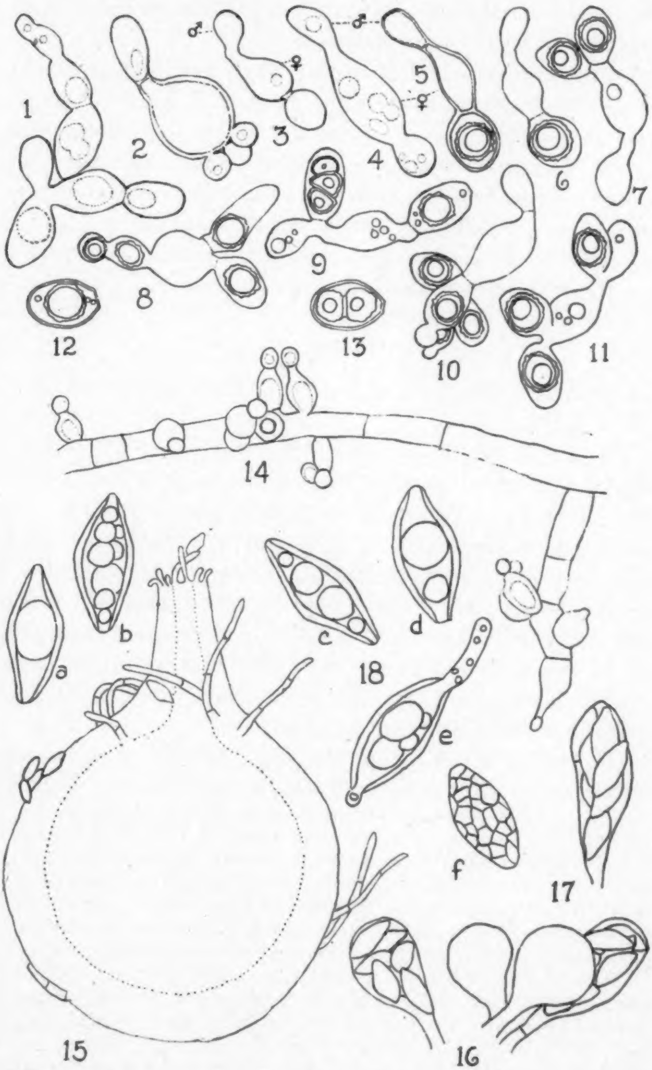

FIG. 1. 1–13, *Nadsonia fulvescens*; 14–18, *Melanospora interna*.

the original description. However, the phialides and phialospores were not reported by these authors.

Two other species of *Melanospora* which have been reported to be associated with *Fusarium* in root-rots are *M. rhizophila* Pegl. & Sacc. in decaying roots of squash and melon, and *M. asclepiadis* Zerova on underground parts of a milkweed. The latter was proved to be parasitic on *Fusarium solani*. Both species are definitely closely related to *M. interna*, but the latter differs from both in several important respects. Apparently neither *M. rhizophila* nor *M. asclepiadis* has been reported in this country.

BASIDIOMCYTES

SIROBASIDIUM SANGUINEUM Lagerh. & Pat. (FIG. 2: nos. 1–11; 4, A)

This fungus was collected during the winter of 1944–1945, in a wooded area behind the Plant Industry Station at Beltsville, Maryland. It was growing on the underside of a decorticated frondose limb lying on the ground. This appears to be the second record of its occurrence in North America. Coker (**1928**) found it in North Carolina. More recently Martin (**1936**) described a specimen of it from Australia. Originally it was described from Ecuador by Lagerheim and Patouillard (**1892**) and is the type species of a new family, based primarily on the seriate nature of the longitudinally or obliquely septate basidia.

The fruiting bodies of the Maryland collection grew in interrupted patches for a distance of about a foot or more. They are

FIG. 2. *Sirobasidium sanguineum* (nos. 1–11). 1, series of three basidia; 2, 3, four-celled basidia, showing oblique, vertical, and transverse septa; 4, top view of a longitudinally septate, four-celled basidium; 5–7, basidia sporulating; 8, four-celled basidium with transverse and oblique septa; 9, three-celled and two-celled basidia in a series, the three-celled one sporulating; 10, four-celled basidium, transversely septate, and two-celled basidium in a series; 11, basidiospores. *Tremella mycophaga* var. *obscura* (nos. 12–25). 12, group of basidia and hyphae among the probasidia of *Dacrymyces* sp.; 13, branched hypha with haustorial branches applied to the *Dacrymyces* hyphae (*a*); 14–16, basidia; 17, basidium sporulating; 18, basidiospores, one of which is producing a secondary spore; 19–21, conidiophores producing conidia; 22, basidium and conidiophore on same hypha; 23, conidia; 24, hypha with clamp connection and haustorial branch; 25, empty basidium of the parasitized *Dacrymyces*. (All drawings × 1173.)

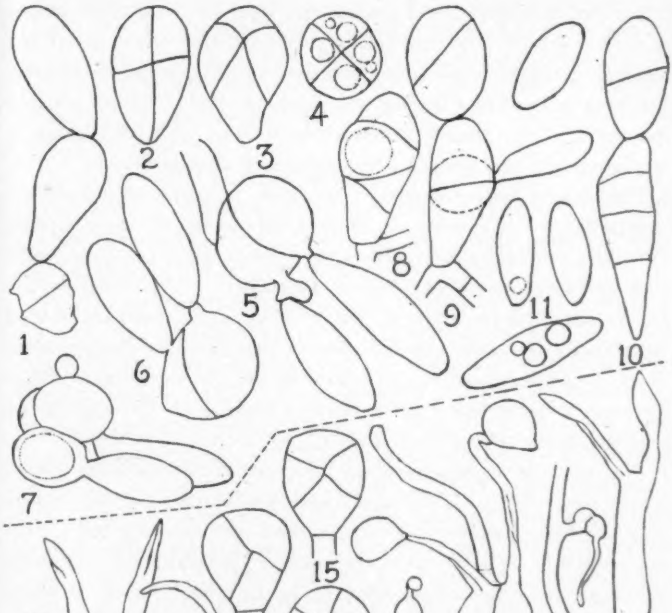

FIG. 2. 1–11, *Sirobasidium sanguineum;* 12–25, *Tremella mycophaga* var. *obscura* on *Dacrymyces minor.*

gelatinous, convoluted and gyrate, and light tan to reddish amber in color. These fructifications are conspicuous when moist but become considerably shrunken and dark reddish brown on drying. The fungus was found during the winter of 1944–1945 but was not sporulating. It was left outdoors until March 9, 1945, when it was examined and found to be sporulating abundantly.

The basidia appear singly or in chains of two to four and are longitudinally, obliquely, or transversely one- to three-septate. They range in shape from oblong or spindle-shaped to nearly globose, and measure 11–12.5 × 12.5–20 (32.5) μ. Basidiospores are mostly rather narrow-elliptic, 5–8 × (11)12–23.3μ, and are budded out directly from the basidial cells or produced on short protrusions, which, however, do not appear to be true sterigmata. Most of these characteristics agree rather closely with those given by Lagerheim and Patouillard, Coker, and Martin.

The extreme variations in basidial types in *Sirobasidium sanguineum* are striking. The basidia are two-, three-, and four-celled, with the two-celled forms predominating and the four-celled next in abundance. The septa may be arranged longitudinally obliquely, or transversely, the oblique septation probably being most common. Four-celled basidia with all three septa transversely arranged are not uncommon. Therefore, from the standpoint of septation, the basidial forms range, in the same fungus, from the *Tremella* type to the *Auricularia* type. It is the occurence of such Heterobasidiomycetes as the present species which tend to emphasize the phylogenetic relationships between tremellaceous and auriculariaceous forms.

TREMELLA MYCOPHAGA Martin var. **obscura** nov. var. (FIG. 2: nos. 12–25)

In fructificationibus *Dacrymycis* parasitica. Conidiis ellipticis, 1.6–3.9 × 3.3–7.8 μ; basidiis subglobosis vel pyriformis, 8–11.7 × 9.8–15.0 μ; basidiosporis 4.2–6.5 × 5.9–9.1 μ.

Parasitic within the fructifications of *Dacrymyces*. Hyphae with clamp connections and numerous haustorial branches with swollen bases which often appear to be composed of double clamp connections. Conidiophores present or absent, usually phialid-like and with basal clamp connections, sometimes occurring on the same

hyphae with basidia, conidia elliptical, 1.6–3.9 × 3.3–7.8 μ. Basidia subglobose to pyriform, with or without basal clamp connections, mostly four-celled, frequently two-celled, the septa vertical to oblique in arrangement, measuring 8–11.7 × 9.8–15.0 μ; basidiospores subglobose to obovate, apiculate, 4.2–6.5 × 5.9–9.1 μ, germinating by repetition.

Parasitic within the fructifications of *Dacrymyces minor* Peck on decorticated frondose wood and *D. deliquescens* Duby on cedar. Deciduous woods on University of Georgia campus, Athens, Georgia, October 23 and 25, 1945.

This fungus was described by me in another paper (**1946**) as *Tremella* sp., parasitic within the fructifications of *Dacrymyces minor* Peck. This first collection was made at Chapel Hill, North Carolina, in March, 1944. No conidiophores or conidia were found in the Chapel Hill specimens, and the fungus was then thought to be most closely allied to *T. tubercularia* Berk., which has been reported as growing from the stromatal cavities of sphaeriaceous fungi, probably as a parasite. Conidia have not been found in the latter species. Moreover, *T. tubercularia* has distinct fructifications, and there are some differences between it and the present species with regard to size of basidia and basidiospores.

Tremella mycophaga, however, possesses conidia and grows on the fructifications of *Aleurodiscus amorphus,* apparently as a parasite (Martin, **1940**). The measurements of basidia and basidiospores vary slightly from those of the new variety, but no outstanding differences were observed. I have examined a specimen of *T. mycophaga* sent to me by Dr. Martin and have found that there is another interesting similarity between the two fungi. The hyphae of *T. mycophaga* give rise to structures resembling the characteristic haustorial branches of the new fungus.

Two important differences exist between these two fungi. *T. mycophaga* possesses distinct fructifications, while the new variety does not; that is, *T. mycophaga* var. *obscura* grows within the fructifications of *Dacrymyces* and has no distinct form of its own. Furthermore, the conidia of *T. mycophaga* are mostly subglobose, whereas those of the new variety tend to be more elliptical. It may be that these differences are sufficiently outstanding to justify the establishment of a separate species for the fungus. However, in

the light of present information, I do not believe that it should be raised above varietal rank.

TREMELLA MESENTERICA (S. F. Gray) Pers.

The fructifications of the specimen at hand are firmly gelatinous, light yellow to bright yellow, mostly with a few flattened and sometimes hollow lobes, and measure 0.8–2 cm. in diameter. Conidia appear abundantly in the hymenium along with basidia which measure 13.6–16.4 × 15.5–23.3 μ. Basidiospores measure 7.8–9.7 × 9.7–13.6 (15.5) μ.

The fungus in several respects resembles very closely descriptions of *T. lutescens* (Pers.) Fries. Coker (**1920**) suggests that *T. mesenterica* and *T. lutescens* may be the same species. At present, the writer accepts the taxonomic treatment of Martin (**1944**) who separates them mainly on the basis of the presence of conidia only in *T. mesenterica*. The latter has apparently not been heretofore reported in North Carolina.

Growing on corticate frondose branches, mountainous area between Old Fort and Bat Cave, North Carolina, July 31, 1945.

DACRYMYCES PUNCTIFORMIS Neuhoff

Numerous, small, light brownish-yellow to brown, gelatinous fructifications of the fungus were found growing on decorticated pine wood. These fructifications were at first pulvinate, but soon become disc-shaped and concave in the center, and some had an irregular, undulate margin. They are attached by a central point and are not confluent. This appears to be our smallest species of *Dacrymyces*, measuring 0.4–1.3 mm. in diameter and drying to small, almost invisible amber-colored or dark brown masses.

Clamp connections are abundant and prominent in this material. Basidia are two-pronged and typical for the group; basidiospores measure 3.9–5.2 × 9–13.5 μ, are reniform, and become one- to three-septate. One fructification was parasitized internally by a *Tremella*-like fungus believed to be *Tremella mycophaga* var. *obscura*, but it was not sporulating.

Collected on old pine wood lying on the ground, mountainous area between Old Fort and Bat Cave, North Carolina, July 15, 1945.

This species of *Dacrymyces* is probably not uncommon in our area, but is easily overlooked because of its small size. There does not seem to be an earlier published report of its occurrence in North Carolina.

GLOEOTULASNELLA PINICOLA (Bres.) Rogers (FIG. 3: nos. 1–9; 4, B)

The fungus was found growing on the under surface of an old oak limb where it covered the bark and some old leather-fungi over a considerable area. The fructifications measure 40–100 μ in thickness and are indefinite in extent, sordid gray to purplish gray in color, gelatinous, and with an undulate or rugose surface. They dry to a thin, black or very dark gray, rough and often carbonaceous layer.

The hyphae are without clamp connections, no gloeocystidia are present, and basidia are mostly in fascicles, forming a rather compact hymenium. They are clavate or clavate-capitate, and measure 8.1–9.6 × 10.4–17.1 μ. The epibasidia are two to four in number, usually four, and measure 6.3–7.2 × 8.1–12.6 μ. The basidiospores are mostly subglobose to obovate, 4.5–5.9 × 5.9–8.4 μ and germinate by repetition.

Collected on *Quercus rubra*, Raleigh, North Carolina, December 10, 1944.

The writer is grateful to Dr. G. W. Martin for his identification of the fungus. The above measurements compare very well with those given by both Martin (**1944**) and Rogers (**1933**). Martin emphasizes the extreme variability of the fungus in nature. This is true with respect to color, texture, and thickness of the fructifications, as well as from the standpoint of microscopic characters. This is apparently the first report of the species from the Southeastern States.

NIDULARIA CASTANEA Ell. & Ev. (FIG. 3: nos. 10–23; 4, C and D)
Granularia castanea (Ell. & Ev.) White.

This interesting fungus has until now been known only from its original locality. It was found growing at Newfield, New Jersey, by Ellis in 1883. White (**1902**) examined the type specimen and described it as *Granularia castanea*. I am following the treatment

of Coker and Couch (**1928**) who prefer to retain the generic name *Nidularia* Fries. They state: "Miss White uses the name *Granularia* Roth, but as Fries and Tulasne use *Nidularia* and the latter clearly defines the genus as now used we retain the latter name. Both names antedate Persoon."

The fungus was found growing in scattered groups on the undersurface of an old piece of rotting canvas along with *Helicosporium aureum* (Cda.) Linder. The latter fungus was identified by Dr. W. W. Diehl. The description of the present collection of *Nidularia castanea* follows:

Peridia small, 0.5–1 mm. in diameter, white, the peridial membrane very thin, almost arachnoid, eventually disappearing and leaving the peridioles bare; peridioles 5–50 in a peridium, chocolate brown, discoid, measuring 0.21–0.42 mm. in diameter, without a funiculus, surrounded by a glutinous substance.

Numerous clamp connections were observed on hyphae of the fungus. Relatively large gelatinizing cells are found in the walls of the peridioles. The hymenium is composed of probasidia, basidia, and a number of variable sterile structures; the latter varying from slender paraphysis-like structures with or without an enlarged, thick-walled base to enlarged thick-walled cells which may represent metamorphosed basidia and whose measurements are $6.3–10.4 \times 9.5–13.5\ \mu$, some probably smaller and easily confused with the larger basidiospores. The basidia are clavate, usually have a slender stalk, sometimes have a gelatinous sheath at the base, and are mostly four-spored, but sometimes two- or three-spored. They measure $4.5–7.2 \times 11.7–26.1\ \mu$. The sterigmata are short or apparently obsolete to conspicuously elongated, and the basidiospores are rather broadly elliptical or obovate, hyaline, thick-walled, smooth, and measure $4.1–6.5 \times 5.9–8.2\ \mu$.

FIG. 3. *Gloeotulasnella pinicola* (nos. 1–9). 1, upright hymenial hyphae bearing basidium and probasidia; 2, 3, probasidia; 4, basidium producing epibasidia; 5, basidium with germinating epibasidia; 6, 7, groups of basidia in various stages of development; 8, epibasidium producing a basidiospore; 9, basidiospores, one of which is germinating by repetition. *Nidularia castanea* (nos. 10–23). 10, group of probasidia, basidia, and slender paraphyses; 11–15, basidia sporulating, those in 14 and 15 with gelatinous sheaths at their bases; 16, thick-walled cells produced in the hymenium; 17, thick walled, long-stalked element presumably produced in the hymenium; 18, 19, parts of the structures with enlarged, thick-walled bases; 20, basidiospores; 21, hyphal fusion; 22, thick-walled gelatinizing cells from the walls of a peridiole; 23, hypha with clamp connections. (All drawings × 945.)

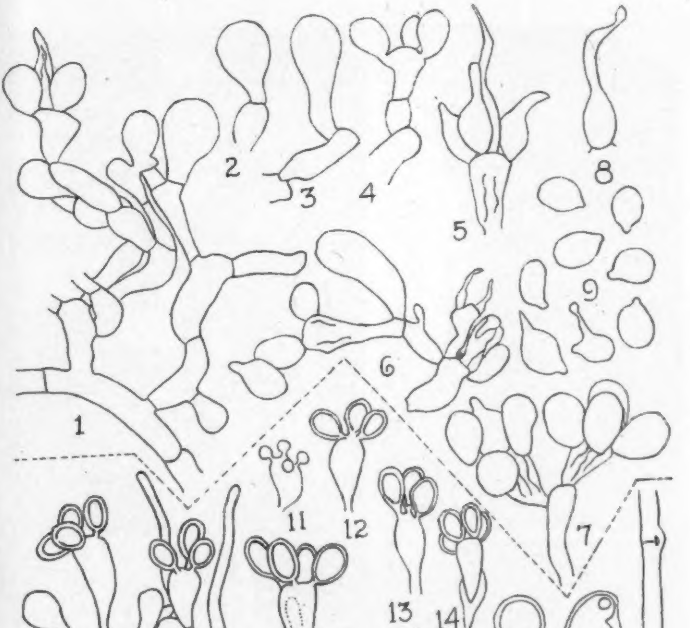

FIG. 3. 1–9, *Gloeotulasnella pinicola;* 10–23, *Nidularia castanea.*

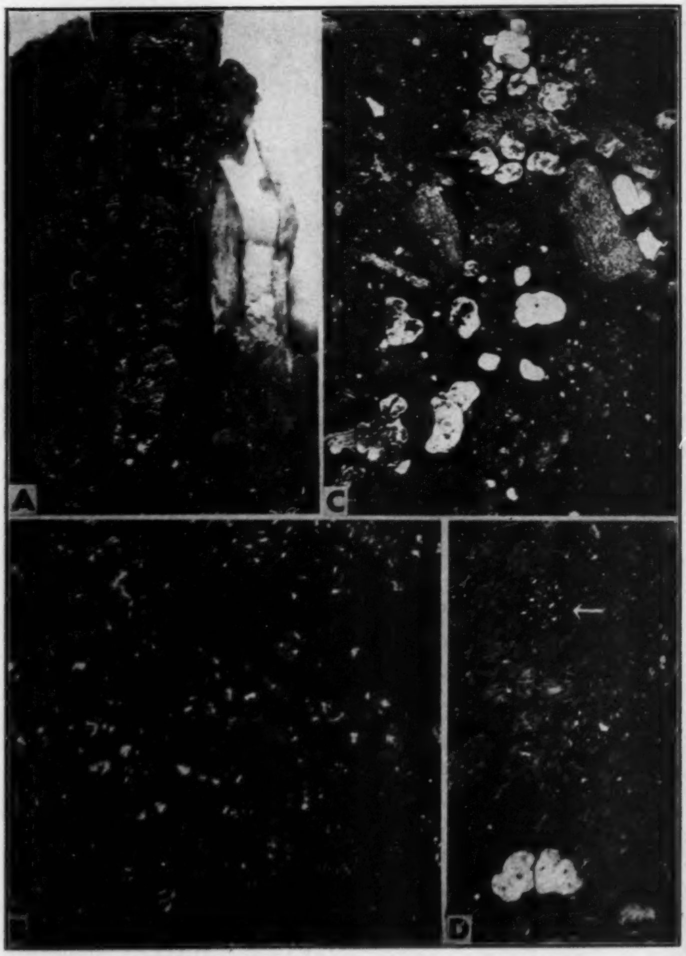

FIG. 4. Photomicrographs. A, *Sirobasidium sanguineum* on piece of dead wood (× 5); B, *Gloeotulasnella pinicola* on bark of dead oak limb (× 10); C, D, *Nidularia castanea*, peridia on rotting canvas (× 8). Note the group of naked peridioles, following the disintegration of the thin peridial wall, in D (arrow).

White gives the spore measurements as $3–6 \times 4–7\,\mu$; whereas Coker and Couch find them to be $4.5–7 \times 6–9\,\mu$. *N. castanea* is easily distinguished from *N. pulvinata* (Schw.) Kuntze, by the much smaller peridia and peridioles of the former. According to Coker and Couch, it is the only other species found in our area.

Collected near the Plant Industry Station, Beltsville, Maryland, May 22, 1945.

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PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI XLII. GORGONICEPS

Fred J. Seaver

(with 2 figures)

The genus *Gorgoniceps* comprises a group of inoperculate cup-fungi in which the apothecia are usually so minute that they are often overlooked by the collector. They are usually rather bright colored or, at least, not very dark, and usually soft and waxy.

Although the apothecia are minute, the spores are often unusually large and resemble those of the genus *Godronia*. However, in *Gorgoniceps* the apothecia are always superficial whereas in *Godronia* they are erumpent. A number of species have been encountered and doubtless there are many more. Those that are known to the writer are listed here.

Gorgoniceps Karst. Myc. Fenn. **1**: 15. 1871.
Belonopsis Sacc. Syll. Fung. **16**: 752. 1902.

Apothecia sessile or short-stipitate, soft and waxy, yellowish or ochraceous, turbinate to subdiscoid or scutellate, seldom exceeding 2–3 mm. in diameter and often less than 1 mm.; asci cylindric to clavate, typically eight-spored; spores elongate, filiform, fusiform, or vermiform, usually becoming multiseptate, hyaline; paraphyses filiform, enlarged above and often branched.

Type species, *Gorgoniceps aridula* Karst.

The genus *Apostemidium* which is usually treated with the Geoglossaceae is close to the present genus and some regard them as synonymous. Durand, however, treated them as distinct and retains the former with the Geoglossaceae because of its general resemblance to *Vibrissea*.

Spores filiform; occurring on bark and cones or leaves of conifers.
Spores 2.5–3 × 65 μ, 16–20-septate .... 1. *G. aridula.*
Spores 2 × 35–40 μ, 3–4-septate .... 2. *G. Pumilionis.*
Spores 1 × 12–15 μ, on leaves of *Pinus* .... 3. *G. ontariensis.*

Spores cylindric fusoid or clavate, occurring on rotten deciduous wood, palm stems and bamboo.

Spores 3–4 × 30–37 µ ....4. *G. iowensis.*
Spores 5–7 × 40–45 µ ....5. *G. confluens.*
Spores 9–10 × 50–55 µ ....6. *G. jamaicensis.*

1. GORGONICEPS ARIDULA Karst. Myc. Fenn. **1**: 185. 1871.

Apothecia gregarious or scattered, sessile or contracted into a very short, stem-like base, bluish-hyaline, when dry pale brownish, reaching a diameter of 0.3–0.8 mm.; hymenium bluish-hyaline or pallid, plane or convex; asci clavate, attenuated above and tapering below into a stem-like base, reaching a length of 100–125 µ and a diameter of 15 µ; spores fasciculate, filiform, straight or curved. becoming septate (the number difficult to determine but apparently 16 to 20), reaching a length of 65 µ and a diameter of 2.5–3 µ; paraphyses filiform about 2 µ in diameter.

On coniferous bark and scales of *Pinus pungens.*

TYPE LOCALITY: Europe.

DISTRIBUTION: Pennsylvania; also in Europe.

ILLUSTRATIONS: E. & P. Nat.-Pfl. **1**^{1}: 208, *f. 163 A;* Rab. Krypt.-Fl. **1**^{3}: *652, f. 1–5.*

The only American specimen of this species seen is one collected by Dr. L. O. Overholts and P. Spaulding (No. *10795*) in Pennsylvania. The plants are minute and the species is probably more common than indicated by the material at hand.

2. GORGONICEPS PUMILIONIS Rehm in Rab. Krypt.-Fl. **1**^{3}: 692. 1896.

Pezicula Pumilionis Rehm, Hedwigia **21**: 115. 1882.

Dermatella Pumilionis Sacc. Syll. Fung. **8**: 490. 1889.

Apothecia gregarious, sessile or contracted into a short, stem-like base, at first rounded, expanding and becoming scutellate, pale cinereous, becoming brownish-yellow, reaching a diameter of 0.1–0.3 mm.; asci clavate, reaching a length of 75–80 µ and a diameter of 6–7 µ, attenuated above and gradually tapering below into a stem-like base; spores filiform, becoming septate (the number of septa usually 3 or 4), 2–2.5 × 35–40 µ; paraphyses filiform, about 1.5 µ in diameter.

On cones of conifers.

TYPE LOCALITY: Europe.

DISTRIBUTION: Colorado; also in Europe.

EXSICCATI: Clements, Crypt. Form. Colo. *290*.

The only American specimen of this species seen is the Clements specimen referred to above on scales of *Picea* sp.

3. GORGONICEPS ONTARIENSIS (Rehm) Hoehnel, Mitt. Inst. Hochs. Wien **3**: 106. 1926.

Pezizella ontariensis Rehm, Ann. Myc. **11**: 167. 1913.

Apothecia scattered, sessile, at first globose expanding becoming cup-shaped, finally discoid, contracted at the base, 0.5–1.5 mm. in diameter, pale yellowish-white, externally floccose; hymenium plane or nearly so, pale rose-colored; asci clavate, reaching a length of 45 μ and a diameter of 6–7 μ, eight-spored; spores filiform, overlapping in the ascus 1 × 12–15 μ; paraphyses filiform, hyaline, 1.5 μ in diameter below, enlarged above to 3 μ.

On needles of *Pinus resinosa.*

TYPE LOCALITY: East Shore of Lake Huron, Ontario.

DISTRIBUTION: Known only from the type locality.

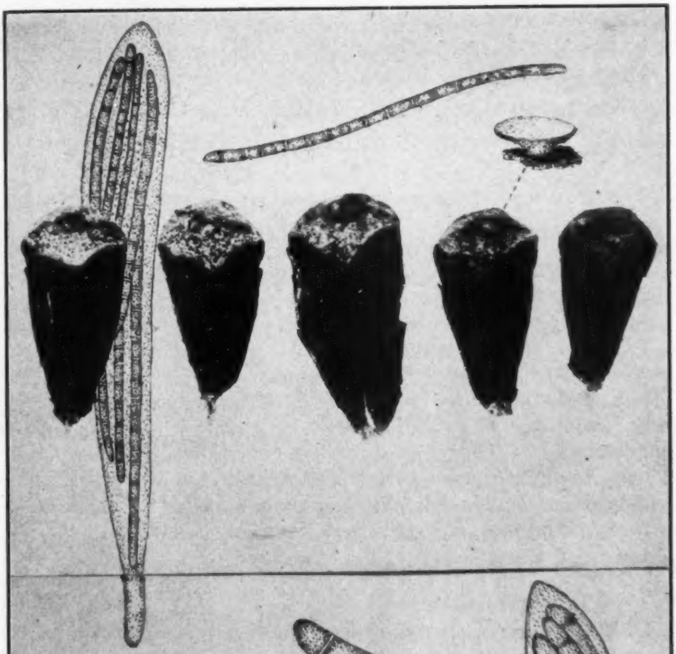
EXSICCATI: Rehm, Ascom. *2030* (apparently part of type material).

4. GORGONICEPS IOWENSIS Rehm, Ann. Myc. **4**: 338. 1906.

Apothecia scattered, at first subglobose, sessile or contracted into a very short stem, expanding and becoming patellate, whitish, with a slight grayish-green tint when dry, pale-brownish, reaching a diameter of 0.2–0.5 mm.; asci clavate, reaching a length of 80–100 μ and a diameter of 10–12 μ; spores subcylindric or clavate, straight or curved, becoming 7-septate, hyaline, 3–4 × 30–37 μ; paraphyses filiform, slightly enlarged above.

Upper figure. *Gorgoniceps aridula.* Photographs of several scales from cones of *Pinus pungens* collected in Pennsylvania by L. O. Overholts. At the left, drawing of an ascus with spores. Above, drawing of one apothecium enlarged; also one spore isolated.

Lower figure. *Gorgoniceps confluens.* Photographs of rotten wood bearing apothecia, with drawings of three apothecia below, much enlarged. At the right, an ascus with spores. Above, one spore isolated. Photographed from type material collected in Bermuda by Stewardson Brown, N. L. Britton and F. J. Seaver in the winter of 1912.



Species of *Gorgoniceps*

On rotten wood.

TYPE LOCALITY: Mt. Pleasant, Iowa.

DISTRIBUTION: New York and Iowa.

ILLUSTRATION: Bull. Lab. Nat. Hist. State Univ. Iowa **6**: *pl. 26, f. 2.*

5. GORGONICEPS CONFLUENS Seaver & Waterston, Mycologia **32**: 399. 1940.

Apothecia gregarious, occasionally crowded and several fusing together, sessile or contracted into a very short, stem-like base, whitish or bluish-white, remaining light-colored or becoming darker when dried, reaching a diameter of 0.5 mm., soft and waxy; hymenium plane or slightly convex, similar in color to the outside of the apothecium; asci broad-clavate, with a very short, stem-like base, attenuated at the apex, reaching a length of 100 μ and a diameter of 14 μ, eight-spored; spores bunched together and overlapping, cylindric, fusoid or subclavate, straight or more often curved or double curved, becoming seven-septate, 5–7 × 40–45 μ; paraphyses filiform, about 2 μ in diameter.

On rotten wood and on palm stems.

TYPE LOCALITY: Bermuda.

DISTRIBUTION: Known only from the type locality.

Type collected in Bermuda by Stewardson Brown, N. L. Britton and Fred J. Seaver (No. *1487*) Nov. 29–Dec. 14, 1912. This is very similar to *G. iowensis* Rehm, which was described from material collected by the author in Iowa. The spores of the Bermuda specimens seem to be larger. Also collected in Paget Marsh on stems of native palm, Seaver & Waterston *62*.

6. **Gorgoniceps jamaicensis** Seaver, sp. nov.

Apotheciis gregariis vel confluentibus, sessilibus vel subsessilibus, subcitrinis, 0.5 mm. diam.; hymenium planum vel concavum; ascis clavatis, 8-sporis, 20 × 140 μ; sporis fasciculatis, subcylindraceis, vel clavatis, 9–10 × 50–55 μ, 7-septatis; paraphysibus filiformibus, 2 μ diam.

Apothecia gregarious or crowded, occasionally several coalescing, sessile or nearly so, becoming patellate, in dried specimens pale yellowish-amber, semitranslucent, reaching a diameter of 0.5 mm.; hymenium plane or slightly concave; asci clavate, eight-spored, reaching a length of 140 μ and a diameter of 20 μ, tapering below

into a short, stem-like base; spores fasciculate, cylindric with the ends attenuated, reaching a length of 50–55 μ and a diameter of 9–10 μ, becoming seven-septate; paraphyses filiform, about 2 μ in diameter.

On bamboo, *Bambos vulgaris*.

Type collected by W. A. and Edna Murrill in Chester Vale, Jamaica, December 21–24, 1908, altitude 3000–4000 ft. (No. *311*).

This seems to differ from our Bermuda species in the much larger spores and asci.

DOUBTFUL SPECIES

Gorgoniceps dinemasporioides (Ellis & Ev.) Sacc. Syll. Fung. **8**: 506. 1889. 1885; *Peziza dinemasporioides* Ellis & Ev. Jour. Myc. **1**: 42. 1885. This was described as a *Peziza* by Ellis and placed in the genus *Gorgoniceps* by Saccardo because of the filiform spores. The spores are not filiform but fusoid and the asci appear to be borne in thin-walled perithecia, clothed with long *Chaetomium*-like hairs. In the opinion of the author this is not a cup-fungus at all.

SOME CYTOLOGICAL OBSERVATIONS ON SPORE FORMATION IN THRAUSTOTHECA CLAVATA

R. K. Saksena and K. S. Bhargava

(with 2 figures)

INTRODUCTION

Spore formation in the family Saprolegniaceae attracted the attention of a number of workers as early as 1850. Almost all of them studied the details of the behavior and the structure of the nuclei and vacuoles in the development of the sporangia and their zoöspores. Guilliermond, Mangenot & Plantefol (**1933**: 295) are the only cytologists who have reported the formation of sporangia in a medium supplemented with neutral red in a species of *Saprolegnia*, and who (Guilliermond **1941**: 62) has been able to follow the entire development of the chondriome in several (*Achlya, Saprolegnia* and *Leptomitus*) living fungi from the germination of their zoospores up to the formation of zoösporangia.

Rothert (**1890**), Hartog (**1887**), Humphrey (**1892**), Davis (**1903**), Weston (**1918**), Schwarze (**1922**) and Murdia (**1939**) have reported a decrease, in general, in the size of the sporangium after the first preliminary division and attributed the shrinkage to the expulsion of cell sap through the sporangium wall. In one of his communications to the senior author, Prof. J. N. Couch wrote, "Of course, no one has actually seen this expulsion. The theory that sap is expelled is based on circumstantial evidence. It is assumed that the sap contains the nutritive juices attractive to bacteria and many observers have noted that when the homogeneous phase starts, bacteria swarm around the sporangium."

The present investigation, therefore, was taken up with a view to study the vacuolar and mitochondrial systems in spore formation in *Thraustotheca clavata* (deBary) Humph.

MATERIAL AND METHODS

The culture of *Thraustotheca clavata* (deBary) Humph. was obtained from Centraal Bureau voor Schimmelcultures, Baarn, Holland.

To obtain sporangia in abundance in several stages desired for study, the methods of Klebs (**1899**) and his successors were tried. The mycelium was grown in a variety of favorable liquid media and then transferred to sterilized distilled water. A more convenient method of obtaining healthier sporangia was to grow the fungus on halves of boiled hemp seed in sterile distilled water. The material for the present investigation was obtained by the latter method.

The development of the sporangium in the living condition and the behavior of zoöspores after liberation were studied in hanging drop cultures with or without neutral red.

For other cytological studies the material was killed and fixed after it had reached the desired stage in development. For the fixation of mitochondria Helley's liquid, Sublimé formol solution and the liquid of Lenhossek were employed (Saksena **1936**: 160). After fixation the material was washed, dehydrated and embedded in paraffin in the usual manner. Sections were cut 4–5 μ thick. Preparations were then stained with iron alum haematoxylin.

The terminology advocated by Weston (**1918**) for the various forms of spores has been used in this paper.

OBSERVATIONS ON LIVING MATERIAL

The development of the sporangium and the liberation of spores in *Thraustotheca clavata* in living condition have been described and figured in considerable detail by Weston (**1918**). Our observations agree with those of Weston and therefore no additional description seems necessary. An important observation to be noted is that most of the mature sporangiospores within the sporangium and those lying close to it show slow undulating movements as in some other members of the family Saprolegniaceae.

INTRAVITAL STAINING: The fungus was grown in small sterilized Petri dishes on halves of hemp seed in sterilized distilled water for about twelve hours. The bits of hemp seed were then transferred to sterilized distilled water to which neutral red had been added

in different concentrations ranging from 0.1–20 mg. per cent, and observations were made with the help of a water immersion lens. No sporangia were developed where the dye was in a concentration of more than 15 mg. per cent. In other cases they were formed and developed to maturity but the number of sporangia decreased with the increased concentration of neutral red. The sporangiospores were discharged only in those cases where neutral red had been added in lower concentrations than 12 mg. per cent, whereas the zoöspores were emitted from the sporangiospores only when the amount of neutral red in the medium, *i.e.*, water, was less than 8 mg. per cent. Guilliermond (**1933**: 295) in a species of *Saprolegnia* found that the sporangia produced zoöspores only in those cases where the concentration of neutral red was less than 5 mg. per cent.

The vacuolar system was studied more closely in a hanging drop culture of a small bit of the mycelium bearing sporangium initials. The mycelium was previously washed in sterilized distilled water to remove the adhering medium containing neutral red. Observations under the high power of the microscope revealed the presence of orange colored axial vacuoles, one in each sporangium initial (FIG. 1, *a*).

In some smaller sporangia, the axial vacuoles are seen to send off small colored extensions towards the wall of the sporangium indicating the beginning of the first preliminary division (FIG. 1, *b*). These extensions later on form a system of intersecting colored lacunae dividing the protoplasm into polygonal masses, which are devoid of any trace of neutral red (FIG. 1, *c*) (first preliminary division). The absence of neutral red solution within the polygonal masses indicates that they do not contain vacuoles.

In the so called homogeneous stage, which next ensues, the color of the dye is seen diffused throughout the sporangium which now shows a slight decrease in its size ($1/15$th of its width). The intensity of the color is now less than that of the original axial vacuole (FIG. 1, *d*). At this stage it seems as if the colored vacuolar sap, set free after the rupture of the parietal membrane of the protoplasm, spreads out, filling up the available space within the sporangium wall. It may be stated here that the colored vacuolar sap is not seen coming out of the sporangium.

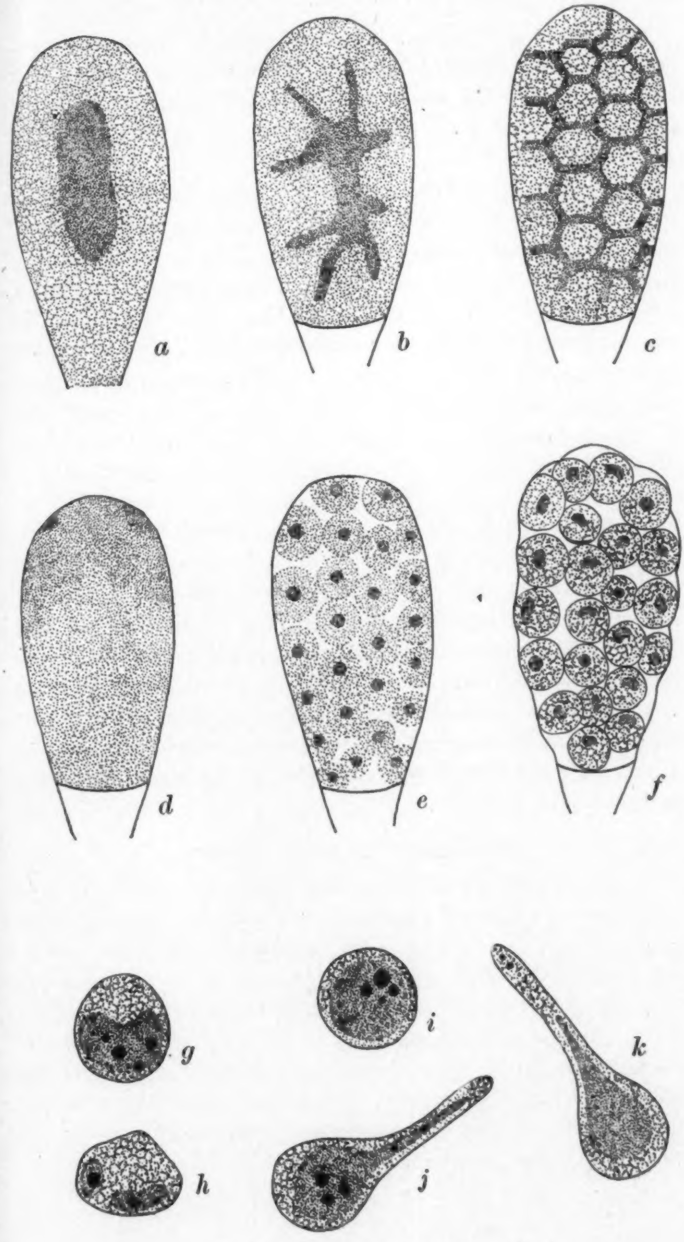


FIG. 1. *Thraustotheca clavata.*

The beginning of the final phase is marked by the rounding up of spore initials, which now appear containing neutral red and showing that each has developed a vacuole within it. At this stage no trace of neutral red is seen outside the spore initials within the sporangium (FIG. 1, *e*).

Finally each spore initial, now transformed into a "sporangiospore," indicates the presence of a cup-shaped (sometimes round) colored vacuole within itself (FIG. 1, *f*). They now come out by bursting the sporangium wall. After a short period a motile zoospore emerges from each sporangiospore. Later on it comes to rest and becomes surrounded by a definite wall. After a time the cystospore, thus formed, begins to germinate giving out a germ tube which later on develops into a hypha. Each zoöspore and cystospore possesses a cup-shaped, occasionally round vacuole containing neutral red (FIG. 1, *h* and *i*). Within the germ tube and the young hypha the vacuole is seen extended into a canal sometimes showing the presence of vacuolar precipitates (FIG. 1, *j* and *k*).

SUPRAVITAL STAINING: The various observations recorded above were noted with this process of staining also. An interesting phenomenon was observed when germinating tubes and young hyphae were treated with neutral red dissolved in Ringer's solution. Besides the vacuolar canal extending from the germinating cystospores, tiny isolated intensely colored vacuoles are seen arising *de novo* at the extreme tips of the germ tubes and young hyphae. They collide and coalesce with each other giving rise to bigger ones by fusion (FIG. 1, *k*).

OBSERVATIONS ON FIXED MATERIAL

Murdia (**1938**) has described and figured the mitochondria in the vegetative hyphae of *Thraustotheca clavata* as long filamentous bodies lying mostly parallel to the longitudinal axis of the hyphae, but we find that they are usually granular at the extreme tips followed by rod shaped and filamentous forms farther back (FIG. 2, *a*). In the sporangium initial in its early stages, the mitochondria are in the form of small rods as well as granules, the filamentous forms being absent. At several places the rod-shaped mitochondria are seen in the process of fragmentation giving rise to their granular form (FIG. 2, *b*).

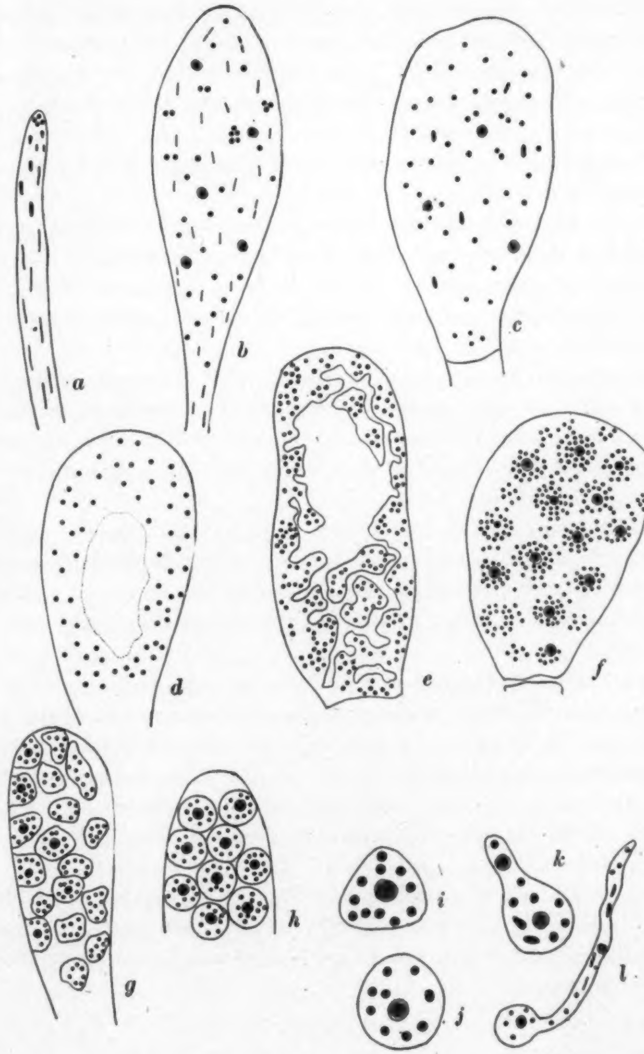


FIG. 2. *Thraustotheca clavata.*

Later on when the septum appears at the base of the young sporangium, mostly granular mitochondria are seen scattered irregularly throughout (FIG. 2, *c*). In a later stage the mitochondria appear shifted towards the periphery, the center of the sporangium being occupied by a vacuole (FIG. 2, *d*). These mitochondria persist in this form in all the later stages of the sporangium (FIGS. 2, *e* to *h*).

With the appearance of a number of irregular clefts arising from the central vacuole, the contents undergo differentiation into a number of spore masses. At this stage of preliminary division, the mitochondria are seen scattered in these roughly polygonal spore masses (FIG. 2, *e*).

During the second phase, *i.e.*, the so-called homogeneous stage, the individual spore masses and the clefts dividing them become much less distinct than heretofore. Now the mitochondria become aggregated around the numerous nuclei, as if the nuclei attracted them strongly (FIG. 2, *f*).

In the stage which next ensues, polygonal spore masses again show their individuality very clearly. These spore origins or spore initials are invariably uninucleate and contain several granular mitochondria more or less aggregated around the nuclei (FIG. 2, *g*).

In mature sporangiospores and also the cystospores the chondriome is exclusively made up of granular mitochondria (FIGS. 2, *h*, *i*, *j*). In the tips of the germ tubes the granular form of mitochondria is maintained (FIG. 2, *j*). In the young hyphae formed by the cystospores rod shaped and filamentous mitochondria are seen behind the tips lying parallel to the longitudinal axis of the hyphae (FIG. 2, *l*).

A prolonged and careful examination of the living hyphae, with and without Janus green Höcht B, revealed that granular forms slowly elongate to give rise to rod shaped and ultimately to filamentous forms.

DISCUSSION

Various authors have reported that there is usually a decrease in the size of the sporangium after the first preliminary division. This shrinkage of the sporangium is said to be due to the expulsion

of the cell sap through the sporangium wall after the splitting of the elastically stretched plasma membrane. Weston (**1918**: 159) in the case of *Thraustotheca clavata* remarks, "we may infer that in the present instance also this rupture takes place, allowing the escape of the cell sap from the clefts with a consequent shrinkage of the sporangium and partial obliteration of the lacunae of demarcation between the spores." Since no one has actually seen this expulsion, we carefully studied the development of sporangia supplied with neutral red intravitally.

At the so-called homogeneous stage there is, no doubt, a slight decrease in the size of the sporangium in *Thraustotheca clavata,* and this must be due to the proportionate diminution of some of its contents. The substance which can escape from the sporangium can be the colored vacuolar sap only, which on being set free after the rupture of the parietal protoplasmic membrane fills up the available space within the sporangium wall. If it is expelled, the color of the neutral red should be visible outside the sporangium wall. We were unable to see this color in the surrounding medium. This may be due to the fact that the small amount of the expelled colored vacuolar sap contains such a minute quantity of neutral red that it becomes imperceptible under the microscope when it diffuses through the surrounding medium. Since at this stage there is a slight decrease in the size of the sporangium, we are at present inclined to think that although only a part of the cell sap may be expelled outside the sporangium wall, most of it is reabsorbed by the developing sporangiospores which are seen containing colored vacuoles as already reported in the foregoing pages, and nothing is left of the colored vacuolar sap outside the young sporangiospores within the sporangium. Later on, the sporangiospores begin to enlarge by absorbing water.

The question as to how the vacuoles originate is a debated one. There are some who believe that they do not arise *de novo* and the general tendency for many years has been to view the *de novo* origin with scepticism. However, the recent work of many authors, specially that of Guilliermond and his students, has conclusively proved that vacuoles may arise *de novo* also. Our observations have shown that the polygonal spore initials before the homogeneous stage do not take up neutral red, thereby showing

the absence of vacuoles within them. Later on with the rounding up of spore initials vacuoles make their appearance, and their presence is at once indicated by the neutral red they contain. Similarly in the tips of the germinating zoöspores vacuoles are seen arising *de novo*. Cassaigne (**1931**) and Guilliermond (**1941**: 180) have reported similar observations in the germinating zoöspores of a species of *Saprolegnia*.

Our studies of the chondriome in this fungus indicate that granular mitochondria exist at the tips of the vegetative hyphae. By elongation they become rod shaped and finally filamentous as found in the regions back of the tip. In the portions of the hyphae which form the sporangium initials, the filamentous mitochondria fragment and give rise to rod shaped forms, which later on become granular by further fragmentation. This form is retained in all the later stages of the sporangium until the germination of the cystospores, in the young germ tubes of which the granular mitochondria at the tips are followed by rod shaped and filamentous forms. Thus our observations support the conclusions of Guilliermond that mitochondria are permanent elements which are found in all parts of fungi and which are never seen to arise *de novo,* and that they are transmitted by division from cell to cell.

SUMMARY

The vacuolar and mitochondrial systems in spore formation in *Thraustotheca clavata* have been studied.

When grown intravitally with neutral red, the young sporangium initial is seen with a colored axial vacuole which later on sends out extensions cutting the protoplasm into polygonal spore initials. The spore initials do not take up the color of neutral red. A homogeneous stage then ensues with the disappearance of the colored vacuolar extensions dividing the spore initials. The spore initials now round up and form sporangiospores each of which now shows the presence of a colored vacuole within it.

Each of the sporangiospores, zoöspores and cystospores presents a cup-shaped or round vacuole within it. Cystospores on germination give rise to germ tubes in the tips of which tiny vacuoles are seen arising *de novo*.

Preparations treated with mitochondrial technique show granular and rod shaped mitochondria in the young sporangium initial. In all the later stages and also in mature sporangiospores and cystospores only the granular form is seen. In the hyphae formed by the germination of cystospores the granular mitochondria at the tips are followed by rod shaped and filamentous forms.

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EXPLANATION OF FIGURES

All the figures were drawn with the aid of camera lucida. The exact magnification of each figure is given at the end of the description. The figures have been reduced one-half in reproduction.

FIGS. 1, *a–f*. Stages in the development of a sporangium grown intravitally with neutral red. *a*. Sporangium initial showing an axial vacuole. × 1100. *b*. Sporangium cut off from the main hypha by a septum and the axial vacuole sending out extensions. × 1100. *c*. Vacuolar extensions forming intersecting lacunae. Protoplasm has become divided into polygonal masses which do not take up neutral red. × 1100. *d*. Sporangium at the so-called homogeneous stage showing diffused color of the neutral red throughout. × 1100. *e*. Spore initials with a vacuole in each. × 1100. *f*. Sporangiospores within a sporangium showing a cup-shaped or round vacuole, one in each. × 1100. *g*. A sporangiospore magnified showing a cup shaped vacuole with vacuolar precipitates. × 2150. *h*. A zoöspore showing two vacuoles with vacuolar precipitates. Cilia are not shown. × 2150. *i*. A cystospore with a round vacuole containing vacuolar precipitates. × 2150. *j*. A germinating cystospore showing the extensions of the vacuole into a vacuolar canal in the germ tube. × 2150. *k*. A germinating cystospore showing the vacuoles arising *de novo* at the tip of the germ tube. × 2150.

FIG. 2, *a*. A hyphal tip highly magnified showing granular mitochondria at the extreme tip, followed by some which are rod shaped. Filamentous mitochondria are seen in the lower part. × 2150. *b*. L.S. of a sporangium intial fixed in Helly's fluid showing nuclei and mitochondria in the form of small rods and granules. Some rod shaped mitochondria are seen in the process of fragmentation. × 1400. *c*. L.S. of a sporangium showing granular mitochondria and nuclei. A few rod shaped mitochondria are also present. × 1400. *d*. L.S. of a sporangium with mitochondria shifted towards the periphery and an axial vacuole. × 1100. *e*. L.S. of the sporangium at the stage of the preliminary division, showing a vacuole in the center and extended lacunae towards the periphery, cutting the cytoplasm into lobes. × 1100. *f*. L.S. of the sporangium at the so-called "homogeneous stage" showing numerous granular mitochondria aggregated round the nuclei. A few are lying scattered. × 1400. *g*. L.S. of a sporangium showing spore initials containing granular mitochondria. In some nuclei are also seen. × 1400. *h*. L.S. of a part of mature sporangium containing sporangiospores. Each sporangiospore contains a nucleus and granular mitochondria. × 1400. *i*. A sporangiospore enlarged showing a nucleus and granular mitochondria. × 2150. *j*. A cystospore showing a nucleus and granular mitochondria. × 2150. *k*. A germinating cystospore showing two nuclei and granular mitochondria. Two mitochondria are seen in the process of elongation. × 2150. *l*. A cystospore with elongated germ tube. A few granular mitochondria are seen at the tips and others which are rod shaped back of them. × 1400.

THE GENUS STOMIOPELTIS (HEMISPHAERIACEAE) [1]

E. S. Luttrell

(WITH 21 FIGURES)

In the vicinity of Experiment, Georgia, in 1942 a hemisphaeriaceous fungus was found causing an olive blotch of the canes of *Arundinaria tecta* (Walt.) Muhl. This fungus appeared to be an undescribed species of *Stomiopeltis*. In order to determine its position in the genus a study was made of all previously described species of *Stomiopeltis*. This study has resulted in a revision of the genus.

TAXONOMY

The genus *Stomiopeltis* was established by Theissen (**1914**) to receive a single species, *S. aspersa* (Berk.) Theiss., which was transferred to the Hemisphaeriaceae from the genus *Calothyrium* in the Microthyriaceae. *Stomiopeltis,* along with two related genera erected at the same time, *Stomiopeltella* and *Plochmopeltella,* was placed in a new subfamily of the Hemisphaeriaceae, the Plochmopeltineae. The Plochmopeltineae were distinguished from the two existing subfamilies of the Hemisphaeriaceae, the Dictyopeltineae and the Thrausmopeltineae, by the presence of a dark-colored superficial mycelium and by the structure of the shield of the ascocarp. This was described as being "hyphis maeandrice sinuosis

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contexta" (Theissen **1914**) or "maeandrisch plectenchymatische" (Theissen and Sydow **1917**). *Stomiopeltis* was distinguished from other genera of the Plochmopeltineae by the presence of paraphyses. Since that time the following six species of *Stomiopeltis* have been described: *S. rubi* (Fckl.) Petr. (Petrak **1923**), *S. cassiae* Mendoza (Stevens and Manter, **1925**), *S. heteromeris* Syd. (Sydow **1927**), *S. philippinensis* Syd. (Sydow and Petrak, **1931**), *S. chilensis* Syd. (Sydow **1932**), and *S. citri* Bitan. (Bitancourt **1934**).

As Bitancourt (**1934**) has pointed out, the vagueness of the description of shield structure in *Stomiopeltis* and the lack of illustration of it have resulted in different interpretations of the genus by later authors and in their inclusion of fungi of diverse structure in *Stomiopeltis*. Examination of material of all species of *Stomiopeltis*, with the exception of *S. philippinensis*, has shown that the species may be divided into two distinct groups. In the first group, consisting of *S. heteromeris, S. chilensis,* and *S. philippinensis*, the shield of the ascocarp is radiate in structure (FIG. 1, 2, 3). At maturity the radiate structure of the shield is somewhat obscured by the curving and twisting of the radiating hyphae and by the irregular lobing of their cells. The tissue thus formed might well be termed "maeandrisch plectenchymatische." Nevertheless, the ascocarps are fundamentally radiate; and, for this reason, the species in this group cannot be retained in the Hemisphaeriaceae. Instead, they must be placed in the Microthyriaceae where their position can be determined only by a review of that family.

In the second group, comprising *S. aspersa, S. rubi, S. cassiae,* and *S. citri,* the shield is non-radiate. It is composed of a pseudoparenchyma of inordinately arranged, sinuous, irregularly lobed cells (FIG. 4, 9, 10, 11). These species are correctly placed in the Hemisphaeriaceae where they form a distinct group deserving generic recognition. The name *Stomiopeltis* is reserved for this group since it includes the species, *S. aspersa,* which Theissen designated as the type of the genus. For reasons considered below under the individual species, *Stomiopeltella suttoniae* Mendoza is transferred to *Stomiopeltis,* the variety *minor* of *S. citri* is raised to specific rank, and the fungus on *Arundinaria* is added to the genus as a new species.

STOMIOPELTIS Theissen, Broteria 12: 73–96. 1914, emend.

Mycelium present at maturity, brown, superficial, reticulated; ascocarps superficial, dimidiate-scutate, ostiolate, uni- or polyloculate, shield composed of a pseudoparenchyma of inordinately arranged, sinuous, irregularly lobed cells, becoming plectenchymatous at the margin and merging with the surrounding mycelial net; asci grouped in locules, more or less prostrate, radiately arranged, their bases lying at the periphery of the locule, their apices converging toward the ostole, pseudoparaphysate; ascus wall thick, composed of two layers; ascospores hyalodidymous.

Type: *Stomiopeltis aspersa* Theiss.

Since no physiological studies have been made on any species of *Stomiopeltis,* it seems best to classify the species strictly upon the basis of comparative morphology and not to recognize as distinct species or varieties morphologically similar forms which occur on different hosts. The majority of the species are rather uniform in structure. In the separation of species variations in dimensions of ascocarps, asci, and ascospores must, therefore, be relied upon. Unfortunately, most of the species are known only from single type collections in which the material is often too scanty or too poorly developed to permit an adequate number of measurements of asci and ascospores. Consequently, it is difficult to define the species satisfactorily; and any arrangement of them in the genus is necessarily tentative.

KEY TO SPECIES OF STOMIOPELTIS

1. Ascocarps containing 2–16 locules....................7. *S. polyloculatis*
1. Ascocarps containing a single locule....................................2
2. Ascocarps over 200 μ in diameter, with a distinct, flat, plectenchymatous border..4. *S. suttoniae*
2. Ascocarps less than 200 μ in diameter, lacking a distinct border.........3
3. Asci oblong to ovoid, less than 30 μ in length..........................4
3. Asci cylindrical to clavate, more than 30 μ in length...................5
4. Ascocarps 64–136 μ in diameter, ascospores hyaline.............2. *S. rubi*
4. Ascocarps 50–80 μ in diameter, ascospores hyaline to yellowish 6. *S. minor*
5. Ascocarps less than 140 μ in diameter.......................3. *S. cassiae*
5. Ascocarps more than 140 μ in diameter...................................6

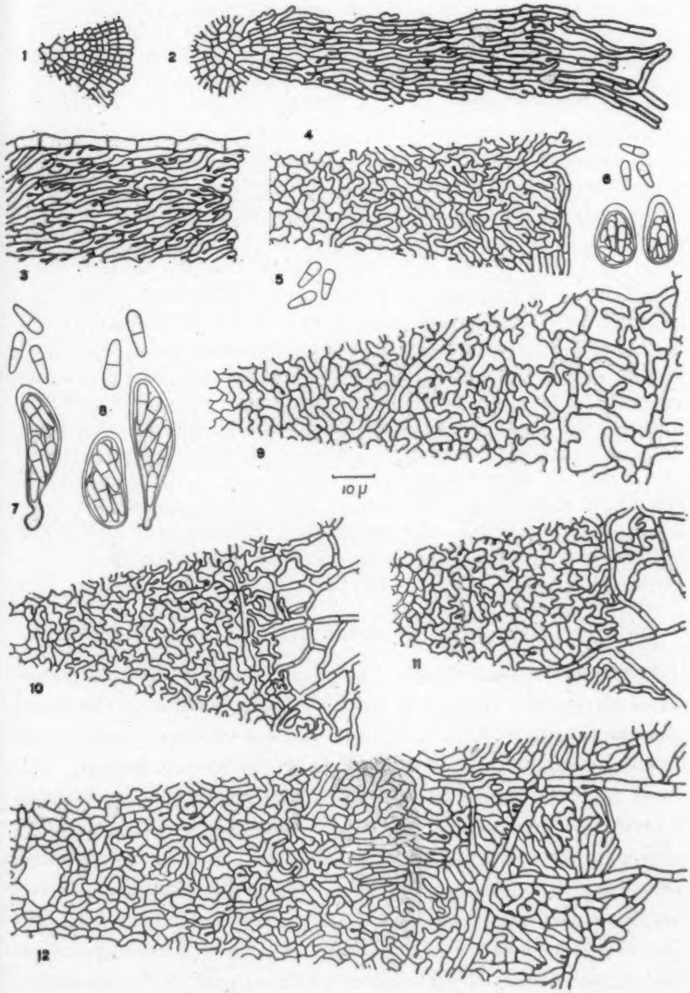
6. Ascospores 6–11 × 2–4 μ .... 5. *S. citri*
6. Ascospores 8–9 × 2–2.7 μ .... 1. *S. aspersa*

1. STOMIOPELTIS ASPERSA (Berk.) Thiess., Broteria **12**: 73–96. 1914.
Asterina aspersa Berk., Decad. No. **476**.
Microthyrium aspersum (Berk.) V. Hoehn., Frag. No. **517**.
Calothyrium aspersum (Berk.) Theiss., Oest. Bot. Zeitschr. **1912**, p. 219.

Mycelium superficial, composed of brown, irregularly branched, anastomosing hyphae about 2 μ in diameter, forming irregular, brownish spots on the lower surface of leaves; ascocarps superficial, dimidiate-scutate, orbicular 137–180 μ in diameter, uniloculate, with a central ostiole; shield (FIG. 4) composed of a pseudoparenchyma of brown, inordinately arranged, sinuous, irregularly lobed cells, becoming plectenchymatous at the margin and merging with the mycelial net; asci pseudoparaphysate, clavate, 35 × 9–10 μ, containing eight irregularly arranged ascospores; ascospores (FIG. 5) hyaline, one-septate, non-constricted, oblanceolate, rounded at both ends, anterior cell shorter and broader than the posterior cell, 8–9 × 2–2.7 μ.

On leaves of a species of Lauraceae in India.

Only two slides, each bearing a single ascocarp, have been available for study. The first was a mount of an ascocarp from the original collection of *Asterina aspersa* in the herbarium of the Royal Botanic Garden at Kew. No asci were present in this mount; and the ascocarp was 285 μ in diameter, which is considerably larger than Theissen's description indicates. It was, therefore, uncertain that it was representative of the material upon which Theissen's description of *S. aspersa* was based. The second slide, obtained from the Farlow Herbarium, was prepared by von Hoehnel and was labelled *Microthyrium aspersum*. The dimensions of the ascocarp in this specimen fell within the limits given by Theissen. Examination of this specimen showed that the shield is composed of an irregular pseudoparenchyma (FIG. 4) and established the fact that this is the type of shield structure which characterizes the genus *Stomiopeltis*. Unfortunately, the few ascospores present in the specimen of *S. aspersa* were, as Theissen stated in his original descrip-



FIGS. 1–12. The genus *Stomiopeltis*.

tion, for the most part immature. The specific limits of *S. aspersa* must, therefore, remain in doubt until additional collections are made.

2. STOMIOPELTIS RUBI (Fckl.) Petrak, Ann. Myc. **21**: 15–16. 1923.
Actinonema rubi Fckl., Symb., p. 384.
Asteroma rubi (Fckl.) Sacc., Syll. Fung. **3**: 202.
Asterella rubi (Fckl.) v. Hoehn., Ann Myc. **1905**, 326.
Asterella rubi var. *rhoina* v. Hoehn., Ann. Myc. **1905**, p. 326.

Mycelium superficial, composed of a network of brown, repeatedly branched, anastomosing hyphae 3–5 μ in diameter, forming sooty-brown spots on stems; ascocarps superficial, dimidiate-scutate, orbicular, 64–136 μ in diameter, uniloculate, centrally ostiolate; shield (FIG. 9) pseudoparenchymatous, composed of brown, inordinately arranged, sinuous, irregularly lobed cells, becoming plectenchymatous at the margin and merging with the mycelial net; asci (FIG. 6) pseudoparaphysate, oblong-ovoid, 16–28 × 7–12 μ, containing eight irregularly arranged ascospores; ascospores (FIG. 6) hyaline, one-septate, non-constricted, oblanceolate, rounded at both ends, anterior cell shorter and broader than the posterior cell, 7–12 × 3–4 μ.

On living stems of *Rubus idaeus* and *Rhois cotinus* in Austria.

Specimens examined were a fragment of *Actinonema rubi* from Thueman's Myc. Univ. No. *1785* in the herbarium of the Royal Botanic Garden at Kew and von Hoehnel's type specimens of *Asterella rubi* and *A. rubi* var. *rhoina* in the Farlow Herbarium. All three of these specimens apparently represent the same species. The shield is composed of an irregular pseudoparenchyma and is similar in structure to that of *Stomiopeltis aspersa.* Petrak was, therefore, correct in transferring *A. rubi* to *Stomiopeltis. A. rubi* var. *rhoina* differs from *A. rubi* only in that it occurs upon a different host (*Rhois cotinus*) and does not form conspicuous spots on the host stems. The latter difference seems to be merely the result of the difference in background furnished by the two hosts. These differences are not considered sufficient to maintain the variety. *A. rubi* var. *rhoina* is, therefore, reduced to synonomy with *Stomiopeltis rubi.*

3. STOMIOPELTIS CASSIAE Mendoza, Bot. Gaz. **79**: 292. 1925.

Mycelium superficial, composed of a network of branching, anastomosing, brown hyphae 1–1.3 μ in diameter, forming faint, olive-colored spots on the upper surface of the host leaves; ascocarps superficial, dimidiate-scutate, orbicular, 88–134 μ in diameter, uniloculate, centrally ostiolate; shield (FIG. 10) pseudoparenchymatous, composed of inordinately arranged, sinuous, irregularly lobed, golden-brown cells, becoming plectenchymatous at the margin and merging with the mycelial net; asci (FIG. 7) pseudoparaphysate (?), clavate, 33–38 × 9 μ, eight-spored, prostrate, radially arranged, their apices directed toward the ostiole; ascospores (FIG. 7) hyaline, one-septate, non-constricted, oblanceolate, rounded at both ends, the anterior cell shorter and broader than the posterior cell, 8–10.5 × 2.6–3 μ (about 13 × 3 μ Mendoza) 2–3-seriate.

On leaves of *Cassia* sp. in British Guiana.

The type specimen (No. *115,* on *Cassia* sp., British Guiana, 10 July, 1922) from the herbarium of the University of Illinois, the Farlow Herbarium and the New York Botanical Garden has been examined.

Although *Stomiopeltis* is distinguished from *Stomiopeltella* by the presence of paraphyses, Mendoza described *Stomiopeltis cassiae* as being aparaphysate. If this were true, the fungus would belong in *Stomiopeltella.* Only a few scattered ascocarps were found in the type material, and it was impossible to determine definitely whether pseudoparaphyses are present. Since, however, this fungus is similar in other respects to species of *Stomiopeltis,* it is retained in this genus until adequate material is available for further study although it must be considered a doubtful species.

Pycnidia were much more abundant on the leaves in the type collection than were ascocarps. These were associated with the ascocarps and superficially were similar to them. The wall of the pycnidium is formed by an irregularly pseudoparenchymatous, ostiolate, dimidiate shield identical in structure with the shield of the ascocarp. Beneath the shield is a hemispherical locule filled with hyaline, cylindrical conidia. These pycnidia are similar to the pycnidia of *S. citri* described by Bitancourt (**1934**) as *Sirothyrium citri* and possibly represent the pycnidial stage of *S. cassiae*. Since the type material is inadequate, additional collections of *S. cassiae*

will be necessary in order to determine its structure satisfactorily and to demonstrate its connection with the associated pycnidia.

4. **Stomiopeltis suttoniae** (Mendoza) comb. nov.
Stomiopeltella suttoniae Mendoza, Bot. Gaz. **79**: 292. 1925.

Mycelium superficial, reticulated, composed of brown hyphae 1.5–4 μ in diameter, forming faint sooty spots on the upper surface of the host leaves; ascocarps superficial, dimidiate-scutate, orbicular, 231–408 μ in diameter, uniloculate, provided with a central ostiole; shield (FIG. 12) differentiated into a convex central portion, 176–231 μ in diameter, composed of a pseudoparenchyma of dark-brown, inordinately arranged, sinuous, irregularly lobed cells and a peripheral, flat, lighter-colored plectenchymatous border 27–95 μ across; asci (FIG. 8) pseudoparaphysate, clavate to ovate-oblong, 30–51 × 12–15 μ, eight-spored, prostrate, radially disposed, their apices converging toward the ostiole; ascospores (FIG. 8) hyaline, one-septate, non-constricted, oblanceolate, rounded at both ends, anterior cell shorter and broader than the posterior cell, 12–15 × 3.5–5 μ, 2–3-seriate.

On leaves of *Suttonia lessertiana* in Hawaii.

The type specimen (No. *1032,* on *Suttonia lessertiana,* Hawaii, 28 July, 1921) from the herbarium of the University of Illinois, the Farlow Herbarium, and the New York Botanical Garden has been examined.

Although Mendoza described this species as being aparaphysate and, therefore, placed it in *Stomiopeltella,* his illustration of the asci (Stevens and Manter, **1925**, FIG. 71) shows them to be paraphysate and the legend to this illustration is "*Stomiopeltella suttoniae.* Fig. 71. Ascus with ascospores and paraphysis." Furthermore, sections of ascocarps from the type specimens have demonstrated that pseudoparaphyses are abundantly present between the asci in the locules. Because it is pseudoparaphysate, this species is transferred to *Stomiopeltis.* In the irregularly pseudoparenchymatous structure of the shield it agrees with other species of this genus. It is distinguished from all other species of *Stomiopeltis,* however, by the broad band of plectenchymatous tissue forming the margin of the shield. It should be noted that the ascocarps in specimens which I have examined are much larger than is indicated in Mendoza's original description. These asco-

carps were, however, abundant on the leaves in the type collection, and no other fungus which could possibly fit Mendoza's description was present. This, together with the fact that my measurements of asci and ascospores agree with those given in the original description, inclines me to believe that the specimens from which my description was taken were representative of the material upon which Mendoza based his description of *Stomiopeltella suttoniae*.

5. STOMIOPELTIS CITRI Bitancourt, Arq. Inst. Biol. São Paulo **5**: 261. 1934.

Mycelium superficial, reticulated, composed of brown hyphae 0.8–3 μ in diameter, forming irregular, effused, grayish-sooty spots on the leaves, stems, and fruits of the host; ascocarps superficial, dimidiate-scutate, orbicular, 140–200 μ in diameter, uniloculate, centrally ostiolate; shield (FIG. 11) pseudoparenchymatous, composed of inordinately arranged, sinuous, irregularly lobed, brown cells, becoming plectenchymatous at the margin and merging with the mycelial net; asci pseudoparaphysate, clavate to cylindrical, 22–46 × 6.5–11 μ, eight-spored, prostrate, radially arranged, their apices directed toward the ostiole; ascospores hyaline, one-septate, non-constricted, oblanceolate, rounded at both ends, anterior cell broader and shorter than the posterior cell, 6–11 × 2–4 μ, biseriate.

Pycnidia (*Sirothyrium citri* Bitancourt) similar to the ascocarps, 80–150 μ in diameter; pycnospores hyaline, cylindrical, rounded at both ends, catenulate, 2.5–6.5 × 0.5–1.2 μ, arising from a hyaline sporogenous layer lining the pycnidium.

On leaves, stems, and fruits of *Citrus* spp. in Brazil.

Specimens (No. *2246, S. Moreira,* 10 June, 1936 and No. *2669, E. Ract,* 17 June, 1937) from the Herbario da Secção de Fitopathologia of the Instituto Biológico de Defesa Agricola e Animal, São Paulo, Brazil, have been examined and deposited in the Farlow Herbarium.

Although there is a difference in the appearance of the ascocarps, the only quantitative difference between *S. citri* and *S. aspersa* is a slight difference in recorded size of ascospores. This difference is of little value since no adequate measurements have been made of the ascospores of *S. aspersa*. These two species are certainly closely related, and possibly *S. citri* should be considered synonymous with *S. aspersa*.

S. citri is the only species of *Stomiopeltis* for which an imperfect stage has been reported. Imperfect stages have, however, been found in a few other species of Hemisphaeriales, and they probably will be found in many others when the species are studied more completely.

6. **Stomiopeltis minor** (Bitancourt) comb. nov.

Stomiopeltis citri var. *minor* Bitancourt, Arq. Inst. Biol., São Paulo **5**: 261. 1934.

Mycelium superficial, reticulated, composed of brown hyphae, forming grayish-sooty spots on the stems, leaves, and fruits of the host; ascocarps superficial, dimidiate-scutate, orbicular, 50–80 μ in diameter, uniloculate, centrally ostiolate; shield pseudoparenchymatous, composed of inordinately arranged, sinuous, irregularly lobed, brown cells, becoming plectenchymatous at the margin and merging with the mycelial net; asci pseudoparaphysate, globose to ovate, 17–28 × 7–12 μ, eight-spored; ascospores hyaline to yellowish, one-septate, non-constricted, oblanceolate, rounded at both ends, anterior cell shorter and broader than the posterior cell, 6–10 × 2–3 μ.

On leaves, stems, and fruits of *Citrus* spp. in Brazil.

This species occurs in association with *S. citri* on the same hosts. Bitancourt appears to have been extremely conservative in considering it merely a variety of *S. citri*. It is more distinct from *S. citri* than are some other species, such as *S. aspersa*. In fact, it seems most closely related to *S. rubi* in its morphology, and perhaps it should be reduced to synonymy with the latter species. For the present, however, it is considered distinct.

7. **Stomiopeltis polyloculatis** sp. nov.

Mycelio superficiali, maculas olivaceas, orbiculares 5–10 mm. diam. vel irregulares et confluentes, tenues efformante, ex hyphis 2–3 μ diam., brunneis, ramoso-reticulatis constituto; ascomate libero, orbiculari, 286–680 μ diam. (medio 446 μ), glabro, dimidiato-scutato, in mycelium reticulatum sensim abuente; contextu scuti brunneo, pseudoparenchymatico, ex cellulis inordinate dispositis, 6–13.5 × 1.5–3.0 μ, irregulariter sinuosis composito, peripherice plectenchymatico; loculis ascigeris numerosis, 2–16 (plerumque 6), poro centrali praeditis; ascis oblongis vel cylindraceis, sessilibus vel breviter stipitatis, 35.0–53.2 × 8.4–13.5 μ (medio 43.96 × 10–22 μ), octosporis, bitunicatis, pseudoparaphysatis, prostratis, radialiter dispositis, apicibus centrum

versus convergentibus; ascosporis 2–3 seriatis, hyalinis, rectis, oblanceolatis, utrimque obtusiusculis, uni-septatis, non-constrictis, superiore loculo breviore ac crassiore, 13.5–21.0 × 4.1–4.8 μ (medio 17.1 × 4.4 μ).

Hab. in culmis vivis *Arundinariae tectae* (Walt.) Muhl. Experiment, Georgia, U. S. A.

The type specimen has been deposited in the Farlow Herbarium, Harvard University, and co-type specimens have been placed in the Mycological Collections of the Bureau of Plant Industry, the herbarium of the New York Botanical Garden, the herbarium of the University of Illinois, and the herbarium of the Royal Botanic Garden at Kew, Surrey, England.

The superficial mycelium and the superficial, dimidiate-scutate ascocarps of this fungus place it at once in the order Hemisphaeriales. Because its shield is non-radiate in structure, it belongs in the family Hemisphaeriaceae. The structure of the shield, a pseudoparenchymatous tissue composed of irregularly lobed, sinuous cells, and the presence of a dark-colored mycelium are characteristic of the subfamily Plochmopeltineae. The characters which show its affinities with the genus *Stomiopeltis* and which separate it from other genera in the Plochmopeltineae are the hyalodidymous ascospores, the presence of pseudoparaphyses and ostioles, and the lack of setae and hyphopodia on the ascocarps and mycelium. Within the genus *Stomiopeltis* it differs from all previously described species in the larger size of the ascocarps and ascospores and in that the ascocarps always contain more than one ascigerous locule.

Inclusion of *S. polyloculatis*, a species with polyloculate ascocarps, in a genus whose species generally possess uniloculate ascocarps might be questioned, especially since variation in number of locules has been employed to some extent in the separation of genera in the Hemisphaeriales (Theissen and Sydow 1917). In some species of *Stomiopeltis* which are usually uniloculate, however, polyloculate ascocarps containing two to five locules may occasionally be found. Since such variation may occur in a single species of the genus, the inclusion of this polyloculate species in *Stomiopeltis* seems justified. Further, it seems doubtful in any case that variation in number of locules in the ascocarp should be considered a suitable criterion for the separation of genera.

EXCLUDED SPECIES

1. STOMIOPELTIS HETEROMERIS Syd., Ann. Myc. **25**: 84–85. 1927. = *Calothyrium* sp. (Microthyriaceae)

A part of the original collection of this species (*Sydow,* No. *169* d, on living leaves of *Phoebe neurophylla,* Costa Rica, 9 February, 1925) in the Mycological Collections of the Bureau of Plant Industry has been examined. The young ascocarps are regularly radiate, resembling those of *Microthyrium* (FIG. 1). In mature ascocarps the cells of the radiating hyphae become irregularly lobed, and the hyphae become curved and somewhat interwoven (FIG. 2). The radiate structure of the shield is still evident, however, as Sydow recognized in his description of this species ("thyriothecia . . . ex hyphis radiantibus sed fortiter undulatis vel fere maeandrice curvatis . . . contexto"). The irregular, undulating appearance of the hyphae does not alter the fact that the shield is fundamentally radiate. *S. heteromeris* is, therefore, transferred to the Microthyriaceae. Because the ascospores are hyalodidymous, it is considered to be a species of *Calothyrium.*

2. STOMIOPELTIS CHILENSIS Syd., Ann. Myc. **30**: 87–88. 1932. = *Asterinella puiggarii* (*Speg.*) *Theiss.* (Microthyriaceae)

A part of Sydow's type specimens (*E. Werdermann, No. 1768,* on living leaves of *Myrtis luma,* Chile, February 1924) from the Farlow Herbarium has been examined. As in *S. heteromeris* the shield is fundamentally radiate in structure (FIG. 3). Sydow, himself, described the ascocarp of *S. chilensis* as being indistinctly radiate ("Strato tegente . . . maeandrice plectenchymatico, ex hyphis . . . indistincte radiantibus maeandrice curvatis constante"). Petrak (**1940**) has previously recognized the fact that *S. chilensis* belongs in the Microthyriaceae rather than in the Hemisphaeriaceae. He considered it a poorly developed form of *Asterinella puiggarii* and reduced it to synonomy with this species.

3. STOMIOPELTIS PHILIPPINENSIS Syd., Ann. Myc. **29**: 248–249. 1931. = species of Microthyriaceae

No specimens of this species have been examined. Sydow, however, described the ascocarp as being in part indistinctly radiate

("strato tegente . . . maeandrice et minute plectenchymatico-cellulosis vel parum elongatis . . . metientibus, partim ex hyphis plus minus fortiter undulato vel maeandrice curvatis saepe etiam indistincte radiantibus . . . constante"). For this reason *S. philippinensis* is tentatively excluded from the Hemisphaericeae. It is referred to the Microthyriaceae although its position cannot be definitely determined until the type specimen is available for study.

MORPHOLOGY OF STOMIOPELTIS POLYLOCULATIS

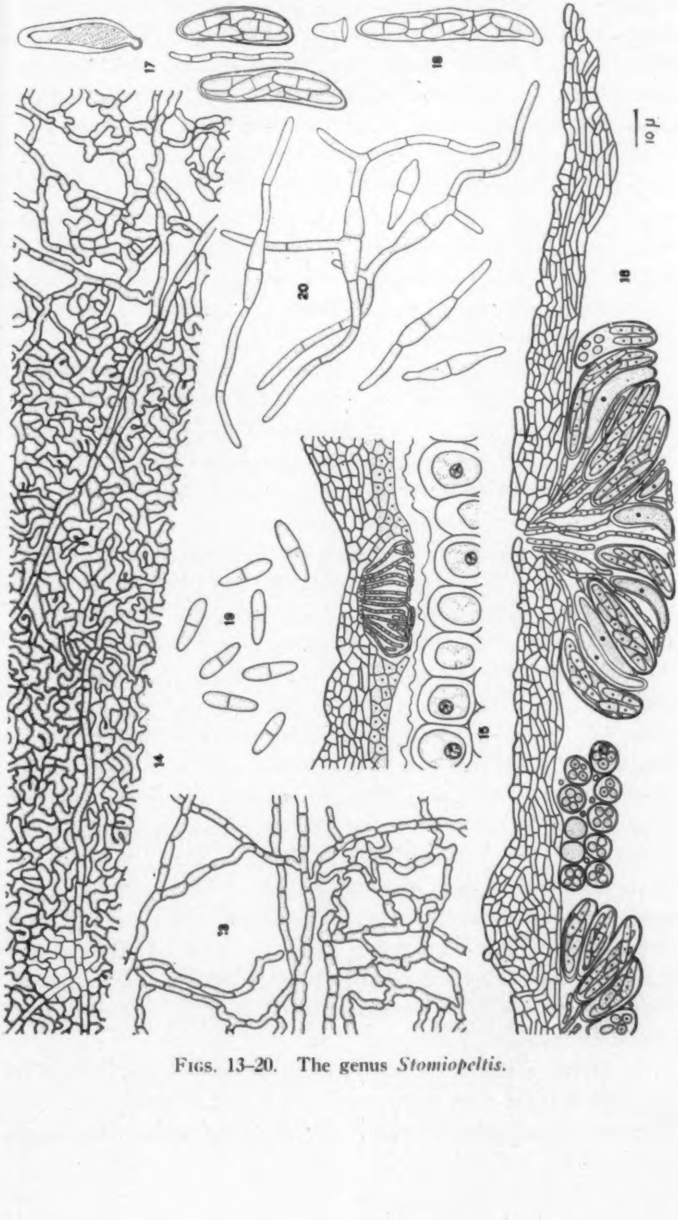
Infection of the current season's canes of *Arundinaria tecta* is accomplished by ascospores during late spring. The resulting mycelium forms a network over the surface of the cane, producing an olive-green blotch. The blotches (FIG. 21) may be circular and five to ten mm. in diameter; or they may spread irregularly, become confluent, and cover considerable portions of the cane. No conidial or spermogonial stage has been observed. During July, however, the young ascocarps appear as black discs, ultimately about 0.5 mm. in diameter, scattered in the mycelium (FIG. 21). Asci are formed within locules in the ascocarps during October and remain in the uninucleate stage during the winter. Ascospores develop during March and April of the following year, and mature ascospores are present from late April through June. These infect the new canes of the host, completing the life cycle of the fungus.

Other undetermined species of *Stomiopeltis* and species of *Microthyriella* frequently are found associated with *S. polyloculatis*. These may be easily distinguished, however, by their macroscopic appearance. The ascocarps of the other species of *Stomiopeltis* are much smaller, being one-fifth or less the diameter of those of *S. polyloculatis;* and, although the ascocarps of the *Microthyriella* species rival those of *S. polyloculatis* in size, they do not appear in blotches such as are characteristic of *Stomiopeltis* species.

The mycelium (FIG. 13) of *S. polyloculatis* is entirely superficial. It rests upon the unaltered cuticle of the canes and does not produce haustoria. The fact that the fungus occurs only upon living canes and that there is no evidence of any external source of food such as sucking insects or their excretions indicates that it is parasitic. Nevertheless, it is difficult to understand how nutrients can be absorbed directly through the thick epidermal walls and heavy

cuticle in the absence of any penetration of the host. The mycelium is composed of brown hyphae 2–3 μ in diameter which branch repeatedly and anastomose freely to form a close reticulum. The cells are short to long cylindrical and may be regular or irregularly lobed and twisted. Hyphopodia are lacking. Ascocarps appear as local thickenings in the mycelium. In the formation of an ascocarp hyphae of the superficial mycelium produce branches which by twisting and branching and by irregular extensions and fusions of their cells fill the interstices of the mycelial net to form a continuous, compact tissue. This is the outer layer of the shield of the ascocarp. At the margin of this flat, orbicular shield (FIG. 14) the hyphal branches are more loosely interwoven to form a plectenchymatous tissue which merges with the surrounding mycelial net. Toward the center of the shield the hyphae, except for the principal hyphae of the mycelium which remain distinct as a network over the surface of the shield, lose their identity; and the tissue formed may best be described as a pseudoparenchyma composed of tortuous, irregularly lobed cells. Seen in surface view, the tissue of Spermatophytes which it approaches most closely is the epidermis, although these fungous cells are often elongated and are even more irregular in outline than are the usual epidermal cells.

Additional layers of cells are added beneath the first formed layer until the shield becomes two to eight cells thick. These cells are thick-walled and brown. Local more opaque spots which appear in the shield in surface view are seen in sections to be produced by small hemispherical thickenings (FIG. 16) which are formed at intervals in the shield. The function of these thickenings is not apparent. A layer of hyaline cells two or three cells in thickness is formed over the lower surface of the shield. These cells are thin-walled and uninucleate. For the most part they become enlarged, and their protoplasm becomes thin; but at intervals groups of cells remain small and retain a denser protoplasm. These cells produce branches composed of small, cylindrical, uninucleate cells which grow downward, their tips directed against the host cuticle (FIG. 15). These hyphae are pseudoparaphyses. By continued growth they raise the portion of the shield immediately above them from the cuticle and create a locule within the stroma.



FIGS. 13–20. The genus *Stomiopeltis*.

Toward the base of the locule the pseudoparaphyses bend outward, expanding the locule centrifugally at the expense of the large, thin-walled cells of the surrounding stroma which are crushed and disintegrated. The tips of the pseudoparaphyses turn inward at the cuticle and intertwine to form a floor to the locule. The asci arise in the base of the locule and grow upward among the pseudoparaphyses. In September, prior to the formation of the young asci, larger, binucleate cells, which probably are ascogenous elements, have been observed among the pseudoparaphyses in the base of the locule. Only a few stages in the development of the ascocarp have been seen, however, and the origin of ascogenous hyphae and asci has not been observed.

From two to sixteen locules are thus formed within the stroma of each ascocarp. The cells in the shield above the center of each locule remain thin-walled and hyaline at the point where each ostiole will form, appearing as translucent areas in the shield. The number of locules may thus be determined in surface view at early stages in the development of the ascocarp.

At maturity the ascocarp consists of a flattened, inverted saucer-shaped shield 286 to 680 μ in diameter covering a number of broadly flask-shaped clusters of pseudoparaphyses and asci (FIG. 16). The asci are more or less prostrate and are arranged radially, their bases lying at the periphery of the locule, their apices converging toward the center. Above the center of the locule the thin-walled cells of the shield disintegrate to form the ostiole. The locules spread out so that they occupy almost the entire space beneath the shield. Often they are in contact with one another. Sometimes a few crushed stromal cells separate them slightly. The asci (FIG. 17) are oblong to cylindrical, $35.0–53.2 \times 8.4–13.5\ \mu$, sessile or short-stipitate, and thick-walled. Each contains eight hyaline, oblanceolate, 2–3-seriate ascospores measuring $13.5–21.0 \times 4.05–4.8\ \mu$. The ascospores (FIG. 19) are one-septate and scarcely if at all constricted at the septum. Both ends are rounded. The posterior cell is, however, slightly longer and narrower than the anterior cell.

At maturity the ascus swells when moistened and it is then apparent that the thick ascus wall is composed of two layers. The thin outer layer splits circumscissilly near the apex of the ascus,

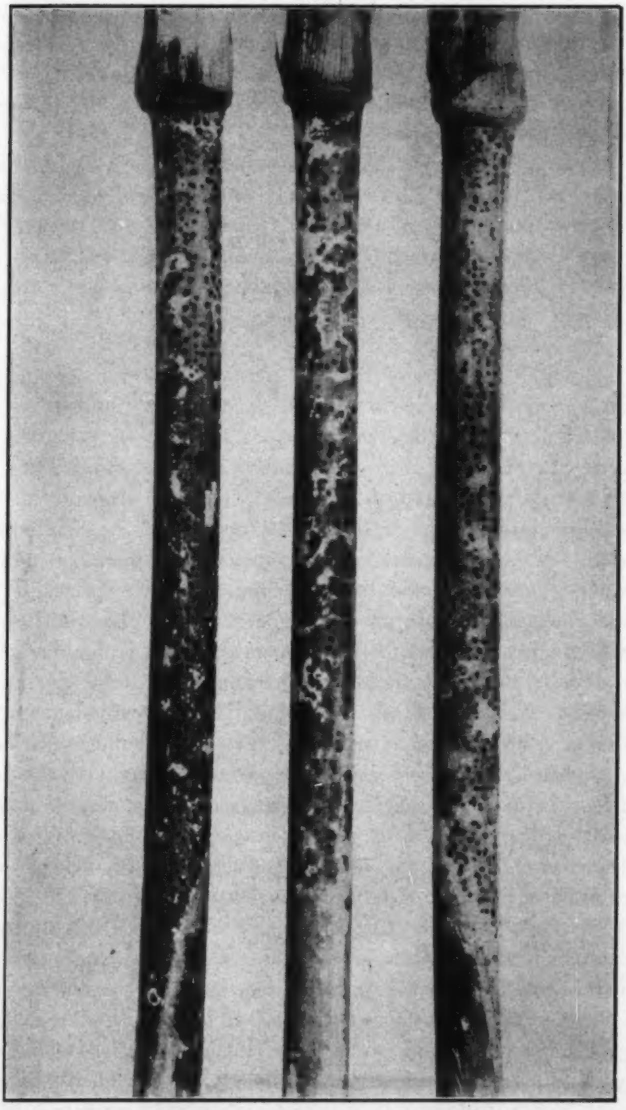


FIG. 21. *Stomiopeltis polyloculatis*, habit photograph showing ascocarps scattered in olive-colored patches on canes of *Arundinaria tecta* × 1⅗.

and the tip is forced off in the form of a small cap (FIG. 18). The thick, gelatinous inner layer then expands to form an elongated sac which protrudes through the ostiole. The ascospores move up into this sac and are forcibly discharged one after another through an elastic pore in the apex.

On germination (FIG. 20) each ascospore produces one or two germ tubes from the sides or, usually, from the end of each cell. The germ tubes elongate and become septate to form a mycelium. On agar media the mycelium forms small, hemispherical, grayish-brown colonies not more than a centimeter in diameter. Further growth in culture has not been obtained.

DISCUSSION

In *Stomiopeltis polyloculatis* the locule in the dimidiate ascostroma is formed by the growth of pseudoparaphyses. These are simple vertical hyphae composed of uninucleate cells and attached at both the top and bottom of the locule. The asci originate in the base of the locule thus formed and grow upward among the pseudoparaphyses. In locule structure *S. polyloculatis* therefore agrees with *Myiocopron smilacis* (De Not.) Sacc. and may be placed in the developmental group previously designated Type 1 (Luttrell **1944**). Study of other genera of the Hemisphaeriaceae has shown that species of *Microthyriella* and of *Schizothyrium* belong in the group included under Type 2 (Luttrell **1944**). These two fundamentally different developmental types occur, therefore, in the nonradiate Hemisphaericeae as well as in the radiate Microthyriaceae. If shield structure and insertion of the ascocarp, characters now employed in the separation of families in the Hemisphaeriales, were considered of minor importance, the order might be divided into two families upon the basis of developmental type. The pseudoparaphysate forms belonging to Type 1 might then constitute a single family; whereas the forms lacking pseudoparaphyses, which are included under Type 2, might be segregated in a second family. If, however, such importance were attached to the internal structure of the locule, the pseudoparaphysate Hemisphaeriales would perhaps be considered more closely related to the Pseudosphaeriales (sensu Miller **1938**) than to the non-pseudoparaphysate members of the same order. For example, if the differences in stromal develop-

ment are disregarded, the structure of the locule in *Myiocopron smilacis* (Hemisphaeriales) is essentially the same as in *Diobotryon morbosum* (S.) Theiss. and Syd. (Pseudosphaeriales) but is quite different from that in *Morenoella quercina* (Ellis and Martin) Theiss. (Hemisphaeriales). It is evident that use of this character as a taxonomic criterion of primary importance would necessitate extensive revision of the Pyrenomycetes. Such changes in the present classification should, however, await more thorough study of development in other orders of the Pyrenomycetes as well as in the Hemisphaeriales.

Since the order Hemisphaeriales itself is founded upon gross morphological characteristics of the ascocarp, the separation of families within the order may well be based upon similar characters. The primary subdivision of the Hemisphaeriales into radiate and non-radiate forms is, for the present at least, a reasonable and practical means of classification although the advisability of further division of the radiate forms upon the basis of insertion of the ascocarp (as in the separation of the Polystomellaceae, and possibly the Stigmateaceae, from the Microthyriaceae) may be questioned. Nevertheless, Petrak (**1929**) criticized the separation of the Microthyriaceae and the Hemisphaeriaceae upon this basis. His objection was that there are many transitional forms which must be described as "undeutlich radiar" and that these forms have been placed in the Hemisphaeriaceae as well as in the Microthyriaceae. *Stomiopeltis heteromeris* and *S. chilensis* are examples of these indistinctly radiate forms. Sydow placed these two species in the Hemisphaeriaceae; but in my opinion, they belong in the Microthyriaceae. The ascocarps of these species are radiate in origin; and their fundamentally radiate structure, although somewhat obscured, is still evident at maturity. If care is exercised in separating such indistinctly radiate forms from the non-radiate forms, it seems that the present division between the Hemisphaeriaceae and the Microthyriaceae may be satisfactorily maintained.

On the other hand, the separation of two of the subfamilies of the Hemisphaeriaceae, the Thrausmopeltineae and the Plochmopeltineae, is uncertain. Theissen and Sydow (**1917**) delimited them as follows: Thrausmopeltineae—no free mycelium, shield pseudoparenchymatous; Plochmopeltineae—free mycelium present, shield

wavy plectenchymatic. Petrak (**1929**) later reduced the type genus of the Plochmopeltineae, *Plochmopeltis,* to synonymy with *Microthyriella* (Thrausmopeltineae); and the type and only species of *Plochmopeltis, P. intricata* (E. & M.) Theiss., became *M. intricata* (E. & M.) Petr. He pointed out that, while the mycelium is better developed in *Plochmopeltis,* a hyaline mycelial net is present in species of *Microthyriella.* He stated further that in shield structure also the two genera intergrade. Examination of the genus *Stomiopeltis* has produced support for Petrak's criticism of the separation of the Plochmopeltineae from the Thrausmopeltineae. A superficial mycelium is present in genera of the Thrausmopeltineae such as *Microthyriella* and *Schizothyrium* as well as in *Stomiopeltis.* The only difference is that in the former it is hyaline and inconspicuous, whereas in the latter it is dark-colored and usually produces spots on the surface of the host. Furthermore, the shield in *Stomiopeltis* is pseudoparenchymatous as it is in genera of the Thrausmopeltineae. *Stomiopeltis* differs only in that the cells composing the pseudoparenchyma are irregularly lobed and sinuous. It appears to be closely related to the genus *Clypeolum* in the Thrausmopeltineae. Further study of the limits of variation in the Thrausmopeltineae will be necessary to determine whether these differences in color of mycelium and in shape of shield cells should be considered sufficient basis for the maintenance of the Plochmopeltineae as a distinct subfamily of the Hemisphaeriaceae.

SUMMARY

Of the seven described species of *Stomiopeltis* (Hemisphaeriaceae) *S. heteromeris* Syd., *S. chilensis* Syd., and *S. philippinensis* Syd., because of their radiate structure, are transferred to the Microthyriaceae.

The four remaining species, *S. rubi* (Fckl.) Petr., *S. cassiae* Mendoza, *S. citri* Bitancourt, and the type of the genus, *S. aspersa* (Berk.) Theiss., form a distinct group in the Hemisphaeriaceae characterized by the non-radiate, irregularly pseudoparenchymic structure of the shield of the superficial, dimidiate-scutate ascocarp, the hyalodidymous ascospores, the presence of pseudoparaphyses and ostioles, and the dark-colored superficial mycelium.

S. citri var. *minor* Bitancourt is elevated to the rank of species and becomes *S. minor* (Bitan.) Luttrell.

Stomiopeltella suttoniae Mendoza, because it is pseudoparaphysate, is transferred to *Stomiopeltis* and becomes *Stomiopeltis suttoniae* (Mendoza) Luttrell.

A fungus found on *Arundinaria tecta* (Walt.) Muhl. in Georgia which differs from all previously described species of *Stomiopeltis* in that its ascocarps are polyloculate is added to the genus as a new species, *S. polyloculatis* Luttrell. The presence of more than one locule in the ascocarp is not considered sufficient basis for the formation of a separate genus.

In *S. polyloculatis* the thick-walled, bitunicate asci develop within locules created in the dimidiate-scutate ascocarps by the growth of pseudoparaphyses.

Presence or absence of pseudoparaphyses might be employed as the primary criterion in the separation of families in the Hemisphaeriales; but in the absence of sufficient data on development of the ascocarps in the Pyrenomycetes, the present classification of the order upon the basis of shield structure should be maintained.

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EXPLANATION OF FIGURES

FIGS. 1–12. Drawings made with the aid of an Abbé camera lucida using a 10X ocular and a 2 mm. objective; all to scale shown in figure 9. 1, *Stomiopeltis heteromeris*, portion of shield of young ascocarp; 2, *S. heteromeris*, portion of shield of mature ascocarp; 3, *S. chilensis*, portion of shield of mature ascocarp; 4, *S. aspersa*, portion of shield of mature ascocarp; 5, *S. aspersa*, ascospores; 6, *S. rubi*, asci and ascospores; 7, *S. cassiae*, ascus and ascospores; 8, *S. suttoniae*, asci and ascospores; 9, *S. rubi*, portion of shield of mature ascocarp; 10, *S. cassiae*, portion of shield of mature ascocarp; 11, *S. citri*, portion of shield of mature ascocarp; 12, *S. suttoniae*, portion of shield of mature ascocarp.

FIGS. 13–20. *Stomiopeltis polyloculatis;* drawings made with the aid of an Abbé camera lucida using a 10X ocular and 2 mm. objective; all to scale shown in figure 16. 13, portion of the superficial mycelium; 14, portion of shield of mature ascocarp; 15, section of locule in young ascocarp showing pseudoparaphyses; 16, section through a mature ascocarp showing several locules beneath the shield; 17, young and mature asci and a pseudoparaphysis; 18, dehiscence of the two-walled ascus; 19, mature ascospores after discharge from the ascus; 20, germinating ascospores.

STUDIES ON THE STRUCTURE OF STREPTOMYCES GRISEUS *

Fernando Carvajal

Studies on the structure of *Streptomyces griseus* have been carried on with the aid of an RCA electron microscope, type "EMB–4," and with a light microscope. Fixed preparations, stained or unstained, were used. Living as well as fixed material was also studied with the aid of a light microscope at magnifications from 100 to 1500 times.

Several strains of *S. griseus* were used including active streptomycin producers and inactive strains. All these strains were isolated by the writer with the exception of three from Dr. S. A. Waksman and one from the American Type Culture Collection.

The following procedure for preparation of aerial mycelium and spore chains for electron microscope studies gave satisfactory results. Material from cultures varying in age from one to seven days old was obtained by lightly touching a platinum loop containing a film of distilled, sterile water to the aerial growth of the organism. The contents on the loop (film of water) are placed on the collodion membrane of the screen and left for a few minutes to dry. The screen is then put in the electron microscope to be observed.

THE MYCELIUM

The active vegetative portion of *S. griseus* is differentiated as a definite mycelium. It is usually less conspicuous than are the fruiting structures. The mycelium is well developed, coenocytic (when young) and well branched usually in a typical monopodial form, straight or wavy. Rarely two or more branches are seen growing from the same place on the main hypha. The basal portion of a new branch is usually constricted and perpendicular to the main hypha (FIG. 2: *B* and *C*). No true septa have been observed in the vegetative young mycelium, but they are sometimes

* Contribution from Scherley Laboratories, Inc., Lawrenceburg, Indiana.

seen in older mycelium. Septa are formed in practically all cases in the delimitations of the reproductive cells. There is more variation in the diameter of the mycelium than in the diameter of the spores. The diameter of the mycelium ranged commonly from 0.3 to 2 microns, rarely more; but usually varied from 0.5 to 1.3 microns. Variations in the diameter of the mycelium were observed even in the same filament, such as thinning or thickening, constrictions, etc. (FIG. 2: *B* and *C*). The hyphal walls are smooth. The young, growing portions of the mycelium are usually dense in protoplasmic contents. Vacuoles were often observed in the cytoplasm of older mycelium.

SPORE FORMATION

S. griseus has a definite aerial sporogenous apparatus. Reproduction is by means of unicellular, asexual spores (conidia) which are produced exogenously in chains on the aerial mycelium and are typically wind borne. Such reproductive cells correspond morphologically to the conidia of the higher fungi (Moniliales).

The spores as formed in the aerial mycelium on solid and liquid media were found to be of various shapes: barrel, oval, bean, spherical, and cylindrical (FIG. 1: *B, C, D, E;* FIG. 2: *A;* and FIG. 3: *D*). The spore dimensions usually fall within the ranges of 0.7–0.9 × 0.7–1.9 microns. In the same spore chain, differences in size and shape were often noted (FIG. 1: *C* and *E*). The behavior of the spore formation was very similar to that reported by early workers in the Actinomycetes (**1**, **2**). The aerial sporogenous hyphae which were often clavate were, at first, continuous and rich in protoplasmic contents. Transverse septa were then laid down simultaneously dividing the structure into uninucleate or multinucleate segments (FIG. 1: *A*). Each cell between two septa increased in size, and constrictions appeared at the septa (FIG. 1: *B*) so that the spores were held in chains and connected to each other by very narrow and fragile isthmuses (FIG. 1: *C* and *E*). In some instances these connecting bridges appeared colorless and appeared to be small, empty tubes (FIG. 1: *E*). The spores increased considerably in size and their cell walls thickened. Sometimes apparently empty spores (which do not stain) were seen in the spore chains.

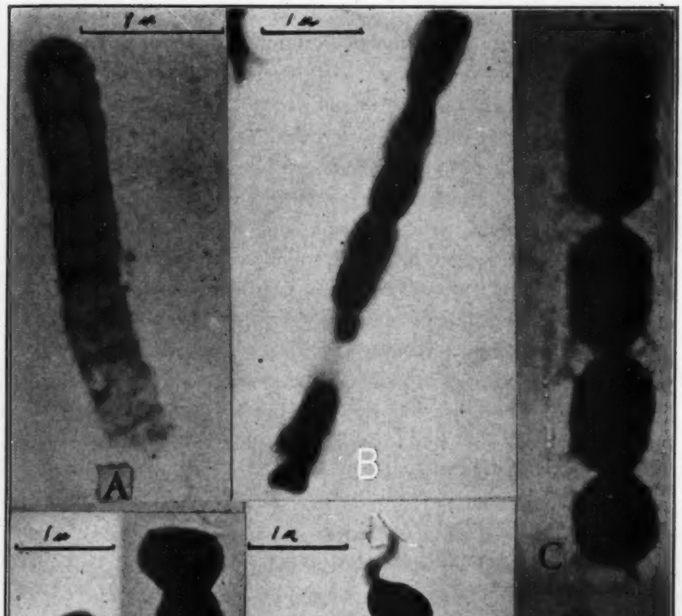

FIG. 1. *Streptomyces griseus.*

Septation sometimes occurred in several branches from an axial filament at the same time whereas, on other occasions with mature spore chains, septate sporogenous hyphae and non-septate filaments were seen in branches from an axial hypha.

In the early stages of spore formation, the aerial mycelium first appears to be whitish to the naked eye; but soon, with the maturation and increase in number of spores, the surface of the culture becomes buff in color, with different tones of green, rose, yellow, orange, gray, cream, and brownish colors according to the nutrient medium used and the strain of the organism. The progressive stages in the development of sporogenous hyphae occurring in *S. griseus* can be easily observed on cultures one to three days old produced on a good sporulation medium (surface seeding).

The aerial, spore-bearing hyphae showed some differences in morphology among *S. griseus* strains growing on the same medium. Differences were found among active strains as well as among inactive ones. The main axial filament was usually branched monopodially. The fertile branches were straight or only slightly wavy. In some strains which sporulate poorly, it was found that comparatively few and scattered sporogenous filaments occurred, especially at the margin of colonies or in concentric rings. These conidiophores or sporogenous hyphae appeared small and often unbranched and were borne directly on the vegetative mycelium, or they were poorly branched with a few short secondary spore chains. On the contrary, strains which sporulate well were seen to have well branched sporogenous hyphae; and the individual spore chain often reached great length. Over 200 spores have been counted on a single spore chain from a culture three days old. The vegetative mycelium gives rise to the aerial mycelium in a monopodial fashion. An aerial filament usually arises at right angles from the horizontal vegetative hypha. Several aerial filaments may arise from the same hypha. Sporogenesis may start at the point of origin of the aerial filament or there may be a short space of sterile hypha.

Mature aerial spores of *S. griseus* often show small fragments of transparent film adhering to the outside of the spores. Figure 3: *D* shows these film particles attached to the exterior of the spores. Figure 1: *D* shows four spores after the film fragments have been

removed by washing and centrifuging several times with distilled water.

SPORE GERMINATION

The spores of *S. griseus* in nutrient broth or solid media usually germinate in a short time at one or both ends. The germ tubes sometimes appear simultaneously, but often one is delayed. The points at which the germ tubes appear are usually the previous points of attachment to other spores or to the hypha. Sometimes the germ tubes arise at various points other than at the ends of the spores. Rarely do the spores germinate by more than two germ tubes. In germination, small protuberances arise at the end or ends of the spores. These elongate to form the germ tube. As the tube elongates by apical growth, the contents of the spore pass into it and the nuclei actively divide; growth and branching of the resulting mycelium follow and finally reproduction starts again. In some instances the original shape of the spore is somewhat modified. Some spores may swell considerably before putting out the germ tubes. Figure 1: *F, G,* and *H* (electron microscope pictures) show three germinated spores of *S. griseus.*

Under favorable temperature and moisture conditions, spores may germinate in a growing culture while still in the aerial chain. Figure 1, *E* shows two germ tubes which originated from the constriction or isthmus which connects the two spores. The germ tubes are emerging at right angles to the main axis of the spore chain. Hyphal fusions were often observed. Germ tubes may fuse with each other.

THE NUCLEUS OF S. GRISEUS

The conspicuous spheroidal and homogenous bodies which are embedded in the cytoplasm are assumed to be the nuclei of *S. griseus*. The fact that they are consistently regular in size, position, and distribution throughout the thallus of this organism, as studied with the light and electron microscopes in many preparations, is taken as basis to support this statement.

The nucleus of *S. griseus* is especially conspicuous and may be demonstrated in the germ tubes, young mycelium, and in the developing spores (FIG. 1: *B, F, E, G, H* and FIG. 2: *A*). The

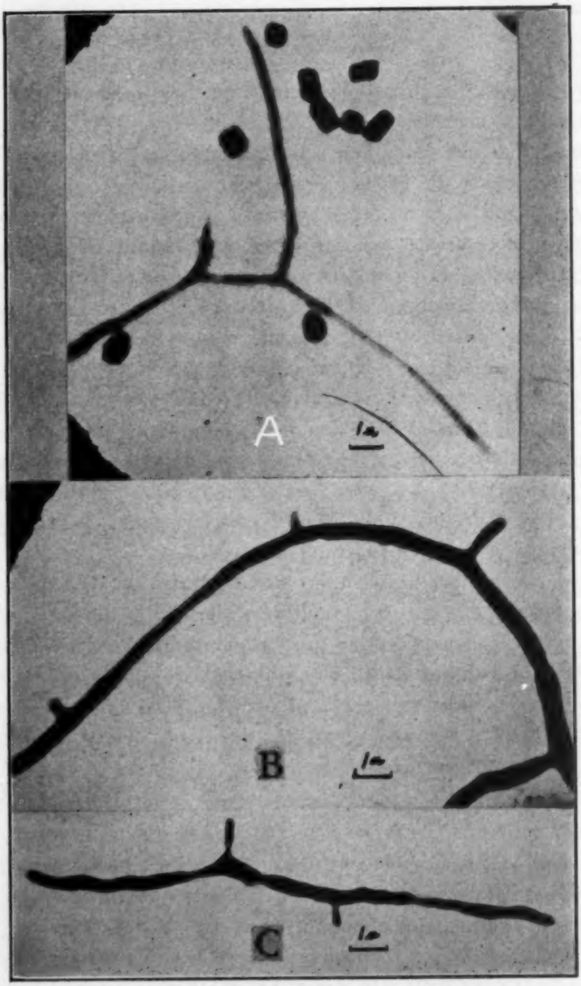

FIG. 2. *Streptomyces griseus.*

nuclei in the mycelium are well distributed throughout the cytoplasm (FIG. 2: *A*) and they move with the cytoplasm. The spores may be uninucleate or multinucleate. The nucleus usually occupies the center of the spore, but may also be found in any other position in the spore.

The number of nuclei is by no means always proportional to the size of the cell; for instance the axial cell at the tip sometimes contains a single nucleus which is slightly larger than the regular sized nuclei in the other cells.

The nuclei are not to be confused here with the metachromatic granules of Neukirch (**3**) and Schütze (**4**).

OTHER ACTINOMYCETES

Other members of the Actinomycetes were also studied. For instance figure 3: *A, B,* and *C* represent spores and spore formation of a chromogenous species of *Streptomyces* which forms spiral spore chains on the aerial mycelium. This *Streptomyces* sp. produces an antibiotic material which is active against gram negative

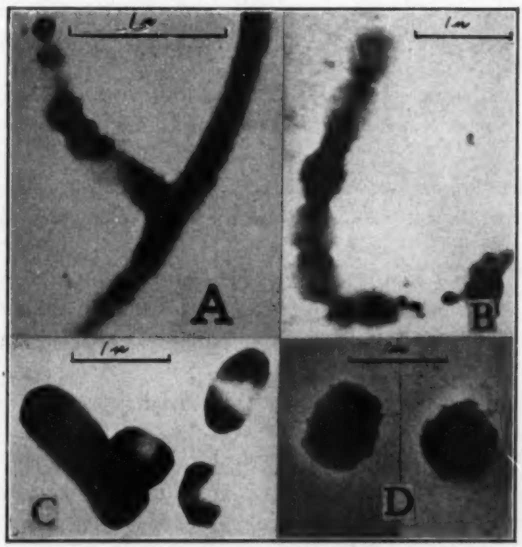

FIG. 3. *Streptomyces griseus.*

and gram positive bacteria. Figure 3: *A* shows an axial aerial hypha with two young spores forming on the side branch; *B* shows a young spore chain, and *C* represents several spores showing differences in shape and size. The spore formation of this organism and others studied was very similar to that of *S. griseus*. The reproductive processes of most *Streptomyces* are far more highly developed than those of any of the bacteria.

SUMMARY

1. Structural studies of active and inactive strains of *S. griseus* were made with light and electron microscopes.

2. The vegetative mycelium when young is coenocytic and well branched typically in a monopodial form. Transverse septa are formed in practically all cases in the delimitation of the reproductive cells. Also septa occasionally were observed in the older mycelium.

3. The basal portions of new mycelial branches were often seen to be constricted.

4. The reproduction of *S. griseus* occurs by means of unicellular, asexual spores (conidia) which are exogenously borne in chains on the aerial mycelium.

5. The spores of *S. griseus* were found to be of various shapes: barrel, oval, bean, spherical, and cylindrical. Differences in shape and size were found often, even among the spores of the same chain.

6. The progressive stages in the development of sporogenous hyphae can be observed easily in a one to three day old culture on a good sporulation media.

7. The aerial sporogenous hyphae showed some differences in morphology among strains growing upon the same medium. Differences were found among active strains, as well as among inactive strains.

8. Mature aerial spores often show small fragments of transparent film adhering to the outside wall.

9. The spores of *S. griseus* usually germinate at one or both ends, usually from the points at which they were attached to the adjacent spores or to the hypha. Rarely do they germinate by more than two germ tubes.

10. Hyphal fusions and germ tube fusions were observed.

11. The nucleus of *S. griseus* may readily be demonstrated in the germ tubes, young mycelium, and in the developing spores. The nuclei are well distributed throughout the cytoplasm of the mycelium. The spores may be uninucleate or multinucleate.

12. Spore formation of other species of *Streptomyces* was very similar to that of *S. griseus*.

13. The reproductive processes of most species of *Streptomyces* are far more highly developed than those of any of the bacteria.

The writer gratefully acknowledges the assistance of Dr. Seth Pope for helpful criticism of the manuscript and of Mr. G. B. Levy and Mr. Denman Shaw for the technical photographic help and the operation of the electron microscope.

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EXPLANATION OF FIGURES

FIG. 1. Electron microscope photographs of *Stroptomyces griseus* SL–842 (streptomycin producer). *A,* an aerial sporogenous hypha showing septation prior to spore formation. *B,* a more advanced stage in spore formation. *C,* a well matured, four-spored chain showing the isthmuses and differences in spore sizes. *D,* four washed spores. *E,* mature spore chain, germinated spores, and isthmuses, showing also an empty isthmus in the lower portion of the chain. *F,* a spore germinated by a single germ tube. *G* and *H,* two spores germinated by two germ tubes.

FIG. 2. Electron microscope photographs of *Streptomyces griseus* SL–842. *A,* shows young branched mycelium with well distributed nuclei; also several spores showing relationship of sizes. *B* and *C,* mycelium from a submerged culture showing branching and irregularities in diameter. Some of the new branches constricted at their bases.

FIG. 3. *A, B,* and *C* are electron microscope photographs of a species of *Streptomyces*. *A,* shows a sporogenous hypha bearing two young spores at the side branch. *B,* a young spore chain. *C,* typical mature spores of various sizes and shapes. *D,* two electron microscope photographs of spores of *Streptomyces griseus* SL–842 showing film fragments adhering to their exterior.

BIOLOGIC STRAINS OF STREPTOMYCES GRISEUS *

Fernando Carvajal

S. griseus was first described by Krainsky in 1914 (**3**) as *Actinomyces griseus,* but according to Drechsler (**2**) this organism is the same species described by Rossi-Doria in 1892 (**4**) as *Streptothrix alba.* Krainsky's original description was amended by Waksman and Curtis (**7**, **8**). Following the recent classification of the Actinomycetes (**9**) this organism is now identified as *Streptomyces griseus* (Krainsky) Waksman and Henrici.

S. griseus has been isolated many times from soil samples, river mud, insects, plant roots, air, foodstuff, animal excreta, water, decomposing plant material, and dust.

The procedure for isolation from soil samples and the testing of *S. griseus* was also used with other microorganisms in the search for antibiotic substances. Soil dilutions employed in isolation work ranged from 1 : 1000 up to 1 : 20,000,000. For material such as fresh river mud, dilutions of 1 : 100,000 up to 1 : 20,000,000 have given good results. The two media most used were of the following composition. 1. Czapek's modified medium: 30.0 gm. sucrose, 1.0 gm. K_2HPO_4, 0.5 gm. $MgSO_4 \cdot 7H_2O$, 0.5 gm. KCl, 0.01 gm. $FeSO_4 \cdot 7H_2O$, 2.0 gm. $NaNO_3$, 15.0 gm. agar per liter of distilled or tap water. 2. Nutrient agar: 10.0 gm. dextrose, 5.0 gm. peptone, 3.0 gm. beef extract, 5.0 gm. NaCl, 15.0 gm. agar per liter of distilled or tap water. The pH of the medium was adjusted with NaOH to 7.5 before sterilization. After the dilution plates were poured, they were incubated at room temperature (70–85° F.) from 8 to 30 days to permit the microrganisms to grow. Organisms from single colonies were tested individually by the author's modification of the "cross-streak agar method" (**1**) for antibiotic activity and pure cultures on agar slants were obtained at the same time.

* Contribution from Schenley Laboratories, Inc., Lawrenceburg, Indiana.

The majority of the strains of *S. griseus* did not produce streptomycin as indicated by the tests using the cross-streak agar method, shake flasks, and aerated bottles. There was no definite indication of relationship in regard to sporulation, growth, pigmentation, and pigment production, etc., in solid and liquid media, between active and inactive strains of this organism.

COMPARATIVE TESTS OF ACTIVE STRAINS OF S. GRISEUS

The cross-streak agar method (FIG. 1) has shown the relation of the intensity of activity at different dates of three active strains of *S. griseus*. They were SL–751 (Waksman's No. 4), SL–841 and SL–842 (the latter two isolated by the author from Ohio River mud) and a *Streptomyces* sp. SL–788 which is a chromogenous type and produces a soluble brown pigment. *S. griseus* SL–842 followed by SL–841, SL–751, and SL–788 respectively exhibits the greatest activity as measured by the size of inhibition zone. Culture SL–788 is an entirely different species of *Streptomyces* and does not produce streptomycin, although it gives a bacteriostatic spectrum very similar to those *S. griseus* strains which produce streptomycin. The bacterial testers gradually grew toward the master streak of SL–788 after the first 24 hours, but the size of the inhibition zones produced by *S. griseus* strains remained the same. Culture S1–788 was later found to produce very little antibiotic material.

Figure 2, *A* shows the bacteriostatic spectrum of *Streptomyces griseus* SL–842 (at center) after four days growth to sixteen different testers (cross-streak agar method). It can be noted in *A* that No. 8 (*S. griseus*) was self inhibited; No. 9 (*Bacillus subtilis,* Turtox) and No. 11 (*Bacillus subtilis* SL–923) were totally inhibited. Figure 2, *B* shows the bacteriostatic spectrum of the same SL–842 against two bacterial testers by the flooding or smearing method (**1**), after four days growth. A gram-negative bacterium was used on one side and a gram-positive bacterium on the other side. It can be noted in *A* that the tester No. 3 (*E. coli* NRRL–B–210) has the same inhibition distance as shown in *B;* the same is true for No. 4 (*Bacillus subtilis* ATCC–6633).

Comparative activity tests using the streak and flooding methods have been run with many other *S. griseus* strains. Tests in sta-

tionary flasks, shaken flasks, and aerated bottle cultures indicated that some strains reached the activity peak in a shorter time than others and that the relative amounts of active material produced varied considerably.

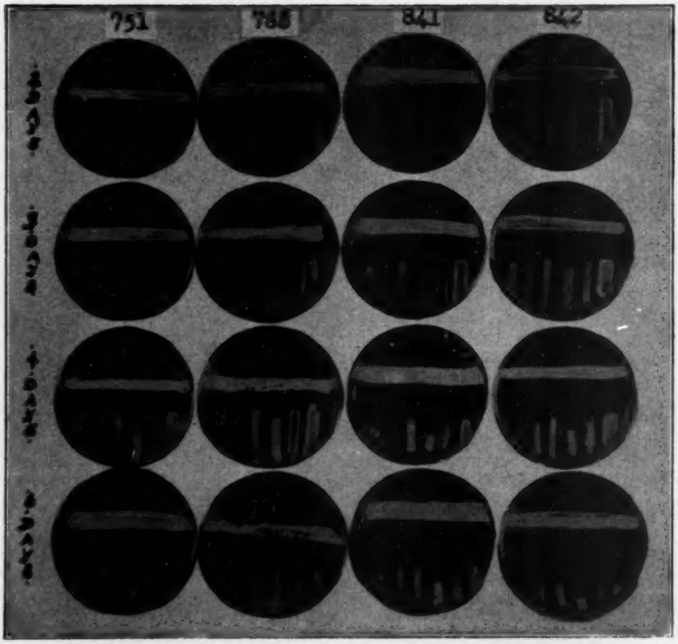

FIG. 1. Comparative bacteriostatic spectra of three active strains of *Streptomyces griseus* to six bacterial testers on different dates. SL–751, SL–841, and SL–842 are three strains of *S. griseus* (streptomycin producers) and No. 788 is a *Streptomyces* sp. Note that *S. grieus* SL–842 is the most active and SL–841, SL–751, and SL–788 follow in that order as measured by the distances of inhibition. The bacterial testers which appear parallel to each other are the same in each plate and are as follows from left to right: *Staphylococcus aureus* ATCC–6538, *Bacillus subtilis* ATCC–6598, *E. coli* NRRL–B–210, *Bacillus subtilis* ATCC–6633, *E. coli* NRRL–B–116, *Bacillus subtilis*, Merck–3R9675.

PROGRESSIVE BACTERIOSTATIC SPECTRUM OF S. GRISEUS SL–842 TO SIX TESTERS—INCUBATION AT ROOM TEMPERATURE (75° F.)

S. griseus was partially inhibited by five bacterial testers (FIG. 3, plate No. 0). Here the master streak and the testers were

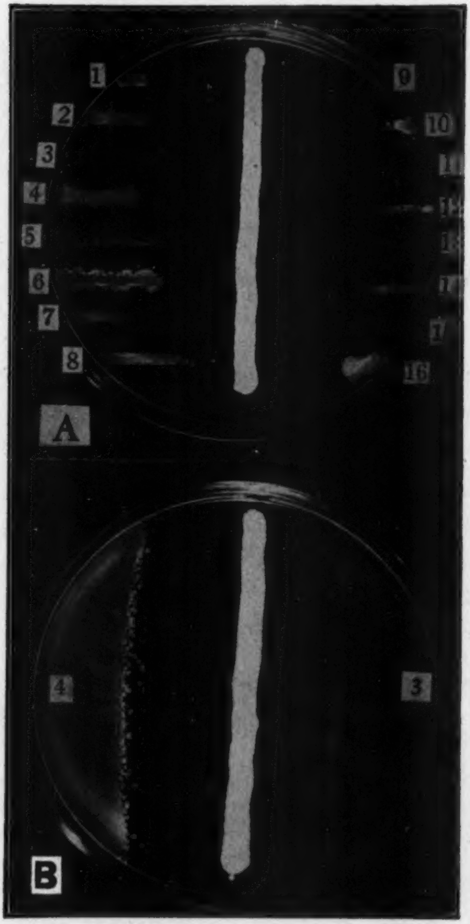

FIG. 2, *A* and *B*. Bacteriostatic spectrum of *S. griseus* SL–842 (at center) after four days growth on large plates (150 × 20 mm.) at room temperature. *A*, cross-streak agar method and *B*, flooding or smearing method. The following testers are indicated by the numbers:

1. *Staph. aureus* ATCC–6538
2. *Bacillus subtilis* ATCC–6598
3. *E. coli* NRRL–B–210
4. *Bacillus subtilis* ATCC–6633
5. *E. coli* NRRL–B–116
6. *B. subtilis* Merck–3R9675
7. *Mycobact. tuberculosis* var. *bovis* ATCC–599
8. *Streptomyces griseus* SL–842
9. *Bacillus subtilis*, Turtox
10. *Mycobact. tuberculosis*, Turtox
11. *Bacillus subtilis* SL–923
12. *Mycobact. tuberculosis* var. *hominis* ATCC–607
13. *Mycobact. Phlei* ATCC–355
14. *Mycobact. tuberculosis* var. *bovis* ATCC–8420
15. *Serratia marcescens*, Turtox
16. *Bacillus mycoides*, Turtox

streaked out at the same time and the results were observed after twenty-four hours. In plate No. 1, *S. griseus* was grown for one day and then the testers were streaked out and results noted after twenty-four hours. *S. griseus* produced enough streptomycin to inhibit for a short distance the growth of all six testers. Note also the slight inhibition of *S. griseus* by three testers at the far left.

The numbers 2, 3, 4, 5, 6, and 7 (on fig. 3) indicate that the testers were streaked out after 2, 3, 4, 5, 6, and 7 days of growth of *S. griseus* and the results recorded after 24 hours. All were incubated at room temperature. Note in them the progressively greater inhibition zone of the testers caused by the higher yields of streptomycin which diffuses out in the agar from the master streak.

OTHER CHARACTERISTICS

It is an advantage for the production of streptomycin to have rapidly growing strains that sporulate well and produce a thick layer of spores on the surface of the agar. Those strains which produce a thicker layer of spore masses usually have much longer and more ramified spore chains and, therefore, a greater number of spores per unit area than poor sporulating strains. The spore masses of some strains when suspended in liquid, such as sterile water or liquid media, form a more or less uniform suspension after good agitation. Other strains on the contrary form very poor suspensions because the majority of the spores will float in clumps on the surface of the liquid and collect at the sides of the container. It has been found that these less wettable spores are usually covered with small fragments of film. This may be due, in part, to the fact that during normal spore production these

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FIG. 3. Progressive bacteriostatic spectrum of *S. griseus* SL–842 (top) to six bacterial testers. Number 0, *S. griseus* and the testers were streaked at the same time. Numbers 1, 2, 3, 4, 5, 6, and 7 indicate that the testers were streaked out after 1, 2, 3, 4, 5, 6, and 7 days of growth of *S. griseus*. The results were observed after 24 hours. The bacterial testers which appear parallel to each other are the same for each plate, and are as follows from left to right: *Staph. aureus* ATCC–6538, *Bacillus subtilis* ATCC–6633, *E. coli* NRRL–B—210, *Mycobacterium tuberculosis* var. *hominis* ATCC–607, *Bacillus mycoides*, Turtox, *Serratia marcescens*, Turtox.

FIG. 3.

strains produce an exudate which, when dried on the outside of the spores, forms a film which may break at several points but still partially adheres to the spores. By constant agitation or warming of the spore suspension, these external particles separate or dissolve from the spores which then go into suspension. These particles, as seen in the electron microscope, appeared as a transparent film. Details of these observations are presented in another publication.

In the course of studies with various media, it was found that the best medium for spore production for one strain may be unsatisfactory for another. Masses of spores of one strain produced on some media gave poor spore suspensions even after good agitation. However, the same strain of *S. griseus* gave a uniform spore suspension with spores produced on another solid medium.

The color and pigment production of strains of *S. griseus* also varies considerably. The aerial mycelium and spore masses of all isolates have a very distinctive buff color with slight variations in intensity. This variation also may be brought about by changing the nutrients, the pH of the medium, temperature, by aging, etc. Some strains produce little or no water-soluble pigment whereas others produce a considerable amount of dark olive-green pigment which diffuses out into the substratum.

The odor produced by strains of this organism grown on organic (plant or animal material) or synthetic media varies considerably. On a given medium, some strains produced a very faint earthy to musty odor whereas others gave a more penetrating odor. The odor was increased markedly in the presence of liver extract.

Variations in the color of the aerial and submerged growth and in the amount of soluble pigment produced are found common to both active and inactive strains. The same is true with the odor produced by these strains.

Colony variability is found among the different strains of *S. griseus* and to a certain extent within groups of colonies derived from the same isolate. Some strains are very stable in their characteristics. Others produce colonies of a rough type with marked and prominent folds, the spore masses of which break up into a chalky powder over the surface of the nutrient agar when dry (FIG. 4, *A*). Other strains give a smooth or fairly flat type of colony of

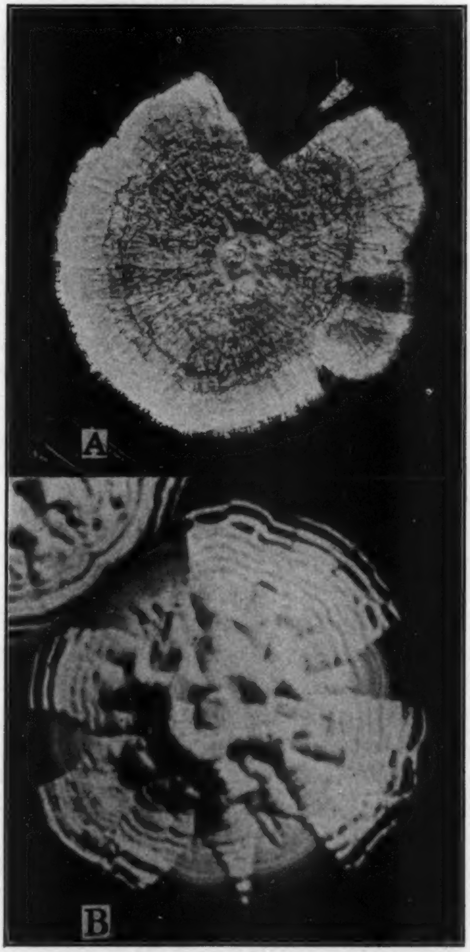

FIG. 4, *A* and *B*. Two colonies of *S. griseus* SL–842 showing different sectors. *A*, is a 3 months old colony reduced half size. *B*, is a one month old colony enlarged 2 times. Note in *B* the concentric rings of growth.

a lighter color which neither forms very extensive aerial mycelium nor has a great amount of sporulation.

Some cultures of *S. griseus* were found to be more unstable than others in their morphological, cultural, and biochemical characteristics. The instability of *S. griseus,* as well as other species of *Streptomyces,* has been demonstrated by several workers (**5**, **6**). The writer, for instance, by making high dilution cultures of spores from *S. griseus* SL–842 obtained three culture types from single colonies which were tested by the cross-streak agar method. They were very similar in cultural characteristics and morphology. The amount of streptomycin and of pigment produced by various strains appeared to be the outstanding differences between them. Most of these strains were equal in activity to the parent culture; a few were slightly less active, and a few were more active than the parent culture. One strain, 842–4, produced less pigment and considerably more streptomycin both in shaken flasks and in deep culture than the parent culture.

By isolating spores from individual sectors produced in isolated colonies or in streak cultures, differences may be found in these cultures when compared with the parent culture. Such variations may be in activity (streptomycin production), rapidity of growth, amount of sporulation, pigmentation, biochemical differences, types of growth (rough or smooth), sterility, etc. Figure 4, *A* and *B* shows two colonies of *S. griseus* which produced sectors of various shapes. Two distinct types, which differed from the parent culture, were obtained from separated sectors of old colonies and from streaks. One type which was about equal in activity to the parent culture produced considerable pigment, a pronounced odor, and heavy sporulation. The other type produced very little pigment, odor, or streptomycin, and sporulation was very light.

CARE AND STORAGE OF CULTURES

Once a pure culture has been obtained, it is necessary to keep it in good condition and ready for use at any time. It is of capital importance to preserve the morphological and physiological as well as biochemical characteristics of the organism, especially those of cultures which produce large amounts of streptomycin.

A general practice used by the author for several years is the following: Fresh agar slants or other containers are seeded by smearing the entire surface with a heavy spore or cell suspension in distilled sterile water. The spores should be from a young, well sporulated culture. This technique has been found advantageous in the culturing of microorganisms such as Actinomycetes, higher fungi, bacteria, and algae. By means of this method the development of individual and separate colonies is very much reduced. When the spores of *S. griseus* germinate on the nutrient agar, the vegetative growth will form an even mat within a few hours. The entire mycelial surface will then work as a single unit and will promptly start sporulation, thus giving maximum production of spores and less vegetative growth in a shorter period of time. The cultures are allowed to sporulate at room temperature (about 75° F.) on a table receiving subdued light. With vigorously sporulating strains of *S. griseus* on a suitable medium, good spore production is obtained in one to five days. After the cultures are well sporulated, they are stored at 3–4° C. In this way, the microorganisms are kept more stable by retarding the appearance of sterile overgrowths, sectorings, variations and mutations. These methods have been found more satisfactory than that of making transfers to agar slants by means of a streak in the center or by seeding at one point.

LYOPHILIZATION OF S. GRISEUS CULTURES

The procedure used was basically that described by Wickerham and Andreasen (**10**). Success has been obtained in the preservation of Actinomycetes. As an example, three different active strains of *S. griseus,* streptomycin producers, were used. Fresh spores were suspended in skimmed milk (made with Difco dehydrated skimmed milk). This suspension after lyophilizing gave an ideal pellet. Several tests for activity, growth, spore production, etc., were made of the lyophilized cultures in comparison with the parent culture, which has been carried on agar slants. Comparative activity tests were made of the three strains by means of the cross-streak agar method, surface, shaken, and aerated bottle cultures. Results showed no difference between the parent and lyophilized cultures. Each strain kept its identity.

SUMMARY

1. The saprophytic fungus *Streptomyces griseus* has been isolated from soils, river muds, insects, plant roots, air, foodstuff, animal excreta, water, decomposing plant material, and dust.

2. The majority of the strains found were unable to produce streptomycin, but a few did.

3. Active strains varied greatly in their ability to produce streptomycin.

4. When *S. griseus* strains were streaked at the same time, perpendicular to various bacterial testers, they were partially inhibited by some of the bacteria, particularly by *Staphylococcus aureus, Bacillus subtilis, and E. coli.*

5. Studies were made of the behavior of several active and inactive strains of *S. griseus*. Variations in growth, sporulation, color, soluble pigment production, odor, and other physiological characteristics were found common to active and inactive strains as well.

6. The best sporulating agar medium for one strain may be unsatisfactory for another strain.

7. Colony variation is found among colonies derived from the same isolate. The active strains may be improved by selection and testing individual colonies.

8. Better stability of cultures was obtained by smearing the whole surface of the nutrient agar medium with a heavy suspension of spores rather than by streaking or seeding at one point.

9. Lyophilized cultures of active strains of *S. griseus* were found not to differ from the parent cultures in morphological, physiological, or biochemical characteristics.

The writer wishes to acknowledge the photographic work which was done by Mrs. Marie Lommel and Mr. G. B. Levy.

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